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Why EPIC should not be squeezed out

DURING April the Science Research Council (SRC) will have to announce some sort of a decision on the future of proposals to build a new high energy physics facility at the Rutherford Laboratory. The machine under consideration is a 14+14 GeV electron-positron intersecting complex called EPIC, and would replace the 8 GeV proton accelerator NIMROD and the 5 GeV electron accelerator NINA—Britain's present major machines. Support for and participation in CERN at Geneva would not be affected by the EPIC proposals.

Almost all accelerators at present in operation generate particles which are fired at stationary targets. As a result of conservation of momentum and of special relativity, most of the energy is wasted in propelling the products of the collision forward. A 400 GeV proton, for instance, fired at a stationary proton provides only 28 GeV of useful energy to the centre-of-mass system. This somewhat underpublicised characteristic of conventional accelerators becomes even less attractive when electrons are the projectiles, and least attractive when electrons are fired at electrons (or positrons). To produce the same 28 GeV in the centre-of-mass system of a conventional electron-positron machine would require 800,000 GeV! Hence high energy physicists have increasingly turned to colliding-beam systems in which two beams of particles travel in opposite direction around a ring and collide at regular intervals. The observer in the laboratory frame of reference then gets full value for money.

But why study electron-positron interactions at all? The scientist not conversant with high energy physics might with some justification ask in an innocent sort of way whether everything hadn't already been thrown at everything else, judging by the great bulk of *Physical Review* and the extraordinary sameness of the titles of papers therein. Apparently not; the more work that is done on protons, for instance, the more complex their substructure seems to be and the more inappropriate proton-proton experiments become for the elucidation of that structure. "It is rather like throwing two watches at each other in order to discover what their internal layout is" was how one high energy physicist described such experiments. But if the trend is then towards using simple particles (electrons are still mercifully believed to be point-like) as probes of complex particles, much more has to be learned about the forces on these simple particles—notably electromagnetic and weak interactions.

The scientific case for EPIC looks good, but scientific cases are hardly the only ingredient at present in decision making in science, particularly where the subject in question is indisputably a 'big science'. The Advisory Board for the Research Councils (ABRC) has recently warned that big science is in for a relatively lean time, and thus the EPIC proposal, which would involve 2,000

man years of staff effort and a capital investment of £25 million to get into operation by 1980, looks particularly vulnerable.

It would obviously make a positive decision much easier if the international dimension were more clearly known. Are there prospects for bilateral or multilateral deals involving cost sharing? It is difficult to assess this at the moment, as other countries, particularly Germany, are also interested in EPIC-type machines, and it is probable that if Britain says no, Germany will build.

The omens are not good. EPIC will be coming up for consideration at the same time as the Northern Hemisphere Observatory proposal; more and more often those involved in big science will find themselves in competition for limited money. It would be easy, and wrong, for the ABRC (which is likely to make the decision) to adopt a Buggins' turn attitude and let the astronomers have their observatory at the expense of EPIC because the astronomers didn't get their last big request, the Mark VA radiotelescope.

It would be equally wrong for the decision to be made in an atmosphere of antipathy to big science in which requests for large sums of money were rejected on some sort of principle that very fine work is being done by people who do not make inordinate demands on resources and it is time to cut the big boys down to size.

Finally, it would be wrong to turn down EPIC on the basis that high energy physicists are an isolated elitist bunch of people who have had a good run for their money, who don't do anything that anyone else can understand and who aren't within sight of any success which is going to provide benefits for the world-at-large. There is some force to these criticisms; high energy physicists are increasingly aware of the alienation between themselves and other scientists and of their general failure to have communicated the intellectual excitement of the subject to a broad audience. Even so, it would be grasping at a convenient but not relevant excuse to make a major decision for this reason.

Morale in the scientific community has never been lower, as job prospects diminish, governmental interest in science touches rock bottom and students turn away from science as a career. A very necessary step to restore some confidence is investment in large-scale long-term projects, and EPIC is just such a one. High energy physics shows strong signs of moving into a period of great excitement, and Britain's previous investments mean that there is much expertise in the field. Support for EPIC would show in an unambiguous way that those who make decisions in British science are aware of the need to restore confidence in pure science as an intellectual exercise, and are prepared to fight for it in high places. □



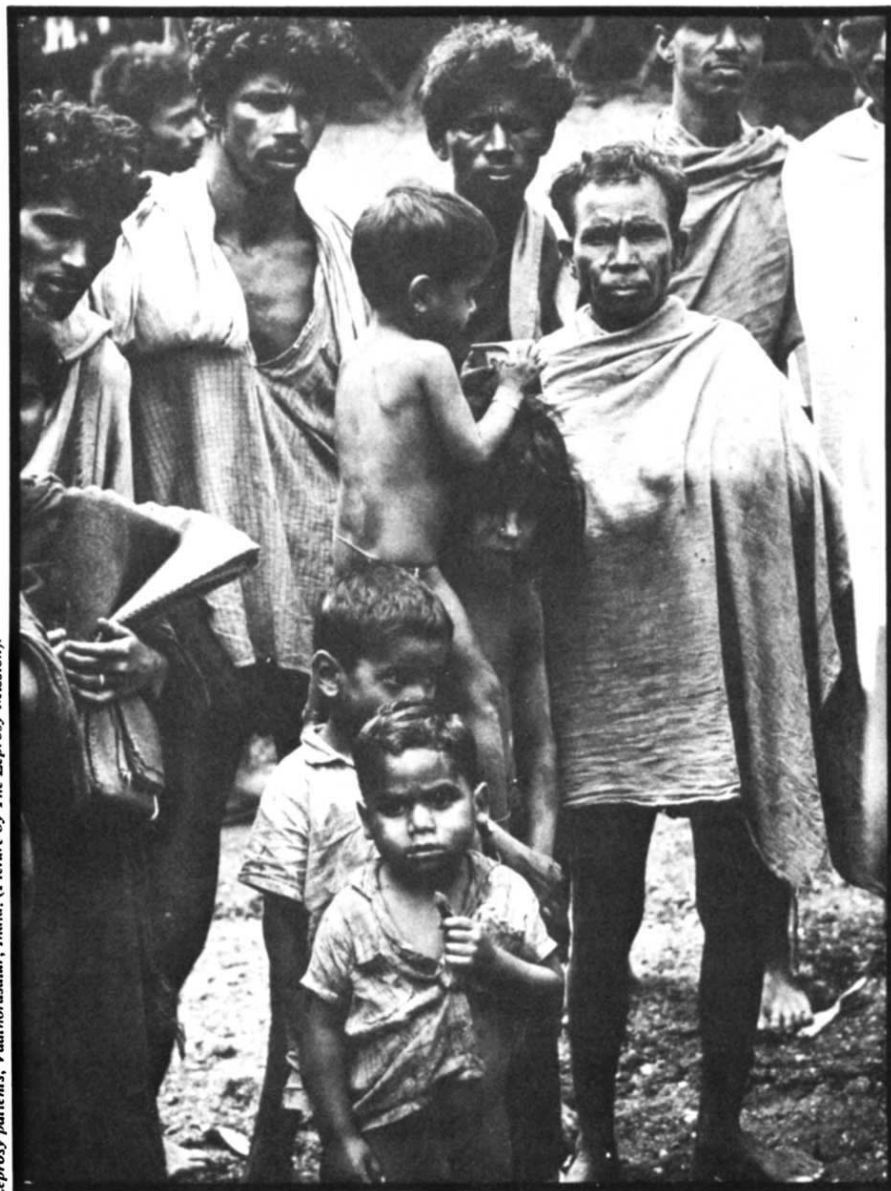
Leprosy: is the strategy wrong?

Authoritative opinion claims that there has been a failure to provide treatment for most of the world leprosy patients and to control the spread of the disease. But sulphones provide a cheap and practical form of treatment and also prevent transmission of leprosy, writes C. L. Crawford, of the Department of Anatomy and Embryology, University College, London.

IN 1873 the Norwegian Armauer Hansen found bacteria in the tissues of leprosy patients and thus established the infectious nature of the disease. Today the World Health Organisation (WHO) estimates there are 10.8 million people with the disease, of whom only about 18% are receiving any treatment. Although information is limited it also concludes (*Bull. Wld. Hlth. Org.*, **46**, 523; 1972) that there has not been any improvement in controlling the spread of the disease over the past five years. The British Leprosy Relief Association (LEPRA) claim that there are as many as 20 million patients. Browne and Davey, two of the most experienced leprologists, (writing this year in *Leprosy Review*), conclude that existing methods have failed to reduce the incidence of the disease, and Ambrose (*Nature*, **248**, 370; 1974) states that leprosy is increasing in South Asia.

Leprologists stress the unknown facts about the disease; the bacteria cannot be cultivated so a vaccine cannot be developed, and the mode of transmission and the incubation period are unknown. Faced with this lack of knowledge and the pessimism of authoritative opinion, the outlook seems hopeless both for success in preventing the spread of the disease and for the patient's chances of obtaining treatment. But the efficacy of the sulphone in providing a cheap and practical form of treatment and in prevention has been grossly neglected.

The difficulty in culturing *Mycobacterium leprae* could, in fact, be seen as a potential advantage in an attack on the disease. *Mycobacterium leprae* is confined to humans and must therefore exist outside the body only for very short periods. There is no known animal reservoir of infection or vector to spread the disease and transmission takes place only between humans. Probably only a proportion of leprosy patients are responsible for



Leprosy patients, Vadhhorasalur, India. (Picture by The Leprosy Mission).

transmitting the disease. This multi-bacillary or lepromatous group may be as low as 10% in some parts of the world and constitutes the reservoir of infection. Also only one in ten of the exposed population, at the most, acquire the disease even in the absence of control measures. If transmission could be interrupted in susceptible people, the disease would decline, as indeed it did in Norway in the last century by a policy of segregation. Also, once eradicated from any country it has never returned. A vaccine is not essential for eradication as the disease has already disappeared from many countries. The transmission of yaws has been successfully interrupted without the bacteria being cultivated or a vaccine developed, by treating patients with penicillin.

The sulphones are the main drugs used in the treatment of leprosy. The experimental multiplication of *M. leprae* in mice is inhibited if *M. leprae* from patients who have received more

than three months of sulphone treatment are injected, suggesting that such bacteria are no longer viable. It may thus be that patients with leprosy are no longer infectious after a few months of treatment, and sulphones may have an important role in preventing the spread of the disease as well as in treatment. Sulphones have been used for the past 25 years and it is essential to determine if they have had any effect in preventing transmission of leprosy.

Any successful preventative measure against leprosy will be shown by a fall in the number of new cases or in the incidence rate; the total number of cases (or 'prevalence' rate) will change much more slowly, because of the inclusion of patients who are already crippled. If the majority are of this type, the prevalence may hardly change at all over a period of years, even though transmission may have been completely interrupted, because leprosy patients can live a long time.

The life expectancy of untreated lepromatous leprosy in Hansen's time was about 10 years; now, with sulphones, it is the same as in the general population. Thus, the 'prevalence' may actually rise in a community treated with sulphones. It is therefore very surprising that the WHO should attempt to draw conclusions about the spread of the disease from the figure of 10.8 million, which is a prevalence estimate. No attempt has been made to obtain information about incidence rates, and even the estimate of the total number of cases is based on very questionable assumptions. Of the 10.8 million, 2.5 million are actually registered; 0.5 million of these are cured or arrested. In each country, to make allowance for unknown cases, a percentage of the registered cases was calculated depending on the efficiency of case finding. This varied between 25% and 300% of the registered cases. This total was added to the 2.5 million registered cases to make up the total of 10.8 million. There must, however, be doubts about the value of figures arrived in such a haphazard way. Where accurate figures of leprosy patients are available, as in Rumania, Cyprus and Libya, it has been shown that the WHO has exaggerated the true figure by from 10 to 50 times. In commenting on the WHO errors in the Cyprus figures, the Cyprus Director-General of Health emphasised that all the patients were old ones and no new cases had occurred in 1970, thus confirming the danger of drawing conclusions about the spread of the disease from prevalence figures. Countries which do have information about incidence rates, such as Okinawa, Hong Kong, New Guinea, Japan and Solomon Islands, Rumania and Cyprus, have all shown a decline. Although their figures will not be completely accurate, they are preferable to the WHO method of assessing the problem.

Most of the countries mentioned have a small leprosy problem. The most accurate information about incidence rates in highly endemic areas has come about in the following way. As no leprosy vaccine is available, BCG vaccine against the similar tubercle bacillus has been used to determine whether it has a protective effect against leprosy. Trials have taken place in Uganda, Burma and New Guinea under the auspices of various research councils, and two trials have also been carried out in India using sulphones for their protective or chemoprophylactic effect by giving them to healthy people. All the trials were controlled by including a group similar in age range which was not protected with BCG or sulphones. At the start of trials the population was sur-

veyed, the leprosy cases were excluded and then new cases were recorded at repeated intervals of between one and two years. Thus, an annual incidence rate can be deduced and the control group will reveal the trend of the disease in these areas, which were deliberately chosen because of the serious leprosy problem.

The results of the BCG trial in Uganda and the two chemoprophylactic trials in India are shown in Tables 1 and 2. All the control groups shows a striking decline in the incidence of leprosy. These two trials took place in communities where sulphones are available to treat patients and, in contrast, the incidence of leprosy in New Guinea did not decline, staying constant at 6 per 1,000 per year from 1964 to 1969; sulphones were not available in this area until late in 1967. Therefore, the most likely explanation of the declining incidence rates is that sulphones given to leprosy patients kill *M. leprae* in a short enough time to interrupt transmission of the disease, thus confirming the experimental findings. The figures for new lepromatous cases are even more dramatic. In two of the areas not a single case was recorded in the controls and in the third the number fell from 11 to 2. Thus, the reservoir of infection has been virtually removed and this makes prospects of eradication bright. The chemotherapeutic and BCG trials were started over ten years ago yet delay in publishing the results fully and in coming to a conclusion about their efficacy has meant that a really effective method of prevention and eradication with sulphones is not being put into practice. If there is still any doubt about the conclusions to be drawn from these trials, these areas

could be resurveyed and the incidence rates recorded in the same age groups as those of ten years ago. There is other evidence based on outpatient incidence rates from two areas of India, Zambia and Uganda and from prevalence rates over a long period in Northern Nigeria, and former French Equatorial Africa (Gabon, Chad, Central African Republic and Congo-Brazzaville). All these have been summarised with my conclusions of the BCG and chemoprophylactic trials previously (*Lancet*, ii 1375, 1971; i, 1186, 1972; *Trop. Doct.*, 3, 137, 1973). Also there is recent evidence of a decline in incidence in Burma and Thailand. Yet Ambrose, Davey and Browne have not mentioned these nor produced any quantitative information to support their own case.

If chemotherapy with sulphones does prevent the spread of the disease then treatment and prevention can be achieved by the same measure, the administration of the drugs to leprosy patients, especially those who are bacteriologically positive. Also by offering to the patients an incentive to attend for treatment, the affected group in the community is automatically brought under supervision, an immense practical advantage over other methods of prevention such as BCG and chemoprophylaxis, which depend on finding healthy contacts of patients. The main sulphone is dapsone which is extremely cheap, costing only 10p a year for each patient, and is easily administered by mouth once a week. Drug resistance is very rare, of the order of 3 per 5,000 lepromatous patients, and it has a wide margin of safety for toxic effects. Thus, mass treatment is practical and economically feasible if given in villages by

Table 1 Annual incidence of leprosy per 1,000 in control and BCG groups in the BCG leprosy trial in Uganda

	Population examined	Control No. with leprosy	Annual incidence	Population examined	Protected with BCG No. with leprosy	Annual incidence
1964	8,071	89	5.5	8,091	18	1.1
1966	9,036	54	3.0	9,052	1	0.05
1968	9,036	31	1.7	9,052	8	0.45

The incidence rate has been halved to give the annual incidence as there is an interval of two years between each survey.

Table 2 Annual incidence of leprosy per 1,000 in Andra Pradesh, India, in controls and chemoprophylactic groups: A, under 25 years of age; B, over 25 years of age

A						
	Population examined	Controls No. with leprosy	Annual incidence	Population examined	Protected with Sulphones (Dapsone) No. with leprosy	Annual incidence
1965	11,270	54	4.79	11,452	29	2.53
1966	12,124	65	5.36	11,900	14	1.17
1967	12,116	37	3.01	12,157	9	0.74
1968	12,931	36	2.78	12,754	3	0.24
B						
	Population examined	Controls No. with leprosy	Annual incidence	Population examined	Protected with Sulphones (Dapsone) No. with leprosy	Annual incidence
1965	8,104	90	11.10	7,851	91	11.59
1966	7,758	67	8.63	7,758	62	7.95
1967	7,337	38	5.17	7,511	45	5.9
1968	7,311	29	3.9	7,282	34	4.6

auxiliary staff who have been trained in diagnosis and treatment.

One area where this approach has been developed most widely is in northern Nigeria and was initiated by Ross. He carried out surveys, trained auxiliary staff and set up outpatient clinics in villages. Patients accepted this approach enthusiastically, whereas before there had been a poor response to treatment based on the leprosarium. In one area, 639 new patients appeared for treatment within 3 months of opening the clinics. There was little fear of stigma against the disease and Ross comments: "There is a degree of shame attached to infection by the disease. Nevertheless, leprosy is tolerated in village and social life and any fear of detection has been associated with the dread of being segregated which means separation from one's family". By 1968 there were 2,000 outpatient clinics throughout the northern region and most patients had access to treatment at an early stage. Unfortunately, few countries adopted such an approach so comprehensively; in many the leprosarium remains the only place where treatment can be obtained, patients have to travel long distances and they fear segregation. The numbers on treatment have remained low for these reasons and not because of lack of money as LEPROA claims in its advertisements. The cost of maintaining patients in institutions is enormously high and measures such as reconstructive surgery have added to the financial burden, making even less money available for work in outpatient clinics. Also, diagnosis is late; patients come late in the course of the disease and thus many are crippled and hence do not benefit from dapsone treatment.

Voluntary agencies in Europe collect more than £2 million annually for leprosy work, but most is still spent on institutional care—in May 1973, for example, the *Sunday Times* published an illustrated article showing the plight of severely crippled leprosy patients in India. At the same time LEPROA appealed for money. £20,000 was collected and in a letter to the *Sunday Times* on December 13, 1973, the Director of LEPROA gave details of how this money was to be spent. All of it was to be used on institutional care, accommodation and food, and on enabling patients to learn a trade such as footwear and cloth making; little money will go to curing and preventing the disease. As most of the patients are already crippled they cannot benefit from dapsone treatment. While there is sympathy for these patients' needs, surely the bulk of the money should go to prevention and cure with sulphones, which LEPROA claims is its aim. □



Accounting for energy

Dr Malcolm Slessor, Director of the Energy Studies Unit, Strathclyde University, sets out the basic principles of energy analysis.

IF economics is the science of scarcity and substitution, energy analysis is no more than the application of economic theory to the one commodity in ultimate limitation on Earth—thermodynamic potential. Perhaps the greatest conceptual difference between economics, as we know it today, and energy analysis lies in the 'system boundary'. Economics sets up a boundary around the system of interest; in energy analysis the system boundary is the whole planet.

Within the framework of economic thought, the economic process will automatically stimulate the development of another energy resource as one resource is depleted and its marginal costs rise. In effect, economics treats the world as a closed system having access to limitless amounts of energy, whose acquisition takes only time, capital labour and technology. On the face of it, it is a good deal easier to justify the position of the economists than that of the energy analysts, but it can be looked at another way.

Any production process, such as building a road or manufacturing pig-iron can only succeed if we inject capital, labour, materials and energy. Considering the properties of these inputs, capital is no more than labour, materials, capital and energy invested at an earlier time. If one goes on to examine every one of the inputs to any process one finds that these inputs are themselves the outcome of a process involving capital, labour, materials and energy. Indeed, the process of network analysis can go back and back until we find that the materials are ores in the ground or produce on the land and the energy is hydrocarbon locked in the

Earth or in deuterium atoms in a highly dilute state in the oceans; labour is mankind and capital has disappeared.

Although both energy and materials are mined and utilised, they have very different features. Materials are never destroyed; the iron molecule in iron ore is still an iron molecule when it is turned into steel and when it ultimately ends up as rust. Moreover, there can never really be a shortage of ores, since the oceans contain very large quantities. We are, however, long past that stage of global development at which ores can be obtained at sufficient rates simply by men labouring with picks and shovels. The energy required to win increasingly depleted ores and turn them into concentrated form will rise to enormous values. It is not the scarcity of the ores that is the problem.

When this thinking is applied to the two examples of making pig-iron and road building, it is certainly conceivable that, given enough poor and willing citizens, a motorway could be constructed by human labour alone. But no amount of human sweat can turn iron ore into iron, or iron into sophisticated machine tools. Though there is a well understood marginal energy cost for labour, so that the two can substitute one for the other to some extent, in the last analysis, energy does what labour cannot do. It carries out processes of transformation involving a decrease in entropy. Energy is irretrievably degraded once it has been used. All energy finishes up as waste heat, and must eventually be dissipated to the surrounding space. Labour, by contrast, is renewable and can also be upgraded through such processes as education and training. Also, as development has proceeded, labour, except in its most skilled forms, has become a surplus commodity in the greater part of the world.

Accordingly, energy analysts believe that it makes sense to measure the cost of the things done, not in money, which is after all nothing more than a highly sophisticated value judgement, but in terms of thermodynamic potential. On the other hand it is widely recognised amongst energy analysts that, as R. S. Berry has stated so succinctly, "if economists in the market place were to determine their shortages by looking further and further into the future, these estimates would come closer and closer to the estimates made by their colleagues, the thermodynamicists".

As energy analysis is concerned with the amount of global energy stock which must be sequestered to make a good or service, it is not enough to assess the amount of oil consumed to heat a house. What is of interest is the total amount of oil in the ground, to-

gether with any other energy sources, that had to be used to make that house heating possible. Thus we also need to know the quantities of energy that were required to win the oil from the ground, bring it from the oil field, refine it and deliver it to the consumer. In the UK these processes add about 18% to the apparent energy requirement for fuel oil for home heating. The error in ignoring the energy requirements for supply are in this case small, because the direct energy costs are the major component. But if we apply the same error to UK agriculture, for example, the direct energy requirements are a mere 42% of the total amount of energy that must in the end have been sequestered in order to make UK agriculture possible. This will have included the energy to make the tractor (duly amortised), pesticides, fertilisers and so on, as well as an appropriate share of the energy to make the machines that make the fertilisers, tractors and so on.

Until August 1974, various practitioners of energy analysis were, of necessity, formulating their own rules. In that month the International Federation of Institutes of Advanced Study (IFIAS) held a workshop in Sweden, attended by twenty people from nine countries, at which the methodology and conventions of energy analysis were thrashed out. Its report is now available (*Workshop Report No. 6*, IFIAS, Stockholm; £4), and the following remarks follow its recommendations.

Perhaps the most fundamental observation is that since our concern with energy is its ability to do work, the calorific or heating values of fuels are not really the quality of interest. Rather it is a more subtle thermodynamic quality known to engineers as 'available work' which in turn closely equals a more easily derivable thermodynamic property, the Gibbs free energy. And indeed in any refined study in energy analysis such as the assessment of waste, for instance, it is the free energy that should be measured.

Nevertheless, the members of the workshop recognised that the calculation of Free Energy would not always be possible and is in any event little understood. For most situations an error of no more than 10% is incurred in taking enthalpy, that is the heating value of a fuel when it combusts with air. This quantity is called the Gross Energy Requirement (GER), normally expressed in megajoules (MJ) or gigajoules (GJ) per unit of output. The workshop defined it as "the amount of energy source or sources which are sequestered by the process of making a good or service". To obtain the 'energy' content, the workshop recommended the convention that it be taken as the

gross heat of combustion with air at 1 bar pressure and 0 °C.

The ramifications of this concept may be gathered from Fig. 1, in which is depicted a hypothetical production process in which natural gas, feed-stock hydrogen and silicon dioxide (from sand) combine to yield a product Y. Suppose that the energy to drive the process comes from coal. There are three system boundaries, of which the inner may be likened to the factory fence, and is essentially the system boundary adopted by company accountants.

The inputs entering through the inner boundary have to be prepared and delivered. Thus silicon dioxide must be refined by (say) acid treatment of sand, hydrogen must be made from natural gas, and natural gas must be brought to the factory fence. These additional processes are depicted by the middle system boundary, somewhat analogous to the nation state. But in the end all the inputs derive from a source in or on the ground—the outer system boundary. In estimating the GER it is essential to go back to this level. Of course, if one's interest is in the energy efficiency of a given sector of a process, then one may choose to examine what happens in the transfer through one boundary to another. This is referred to as the Process Energy Requirement.

Another valuable permutation is that of Net Energy Requirement (NER).

Fig 1: hypothetical production process

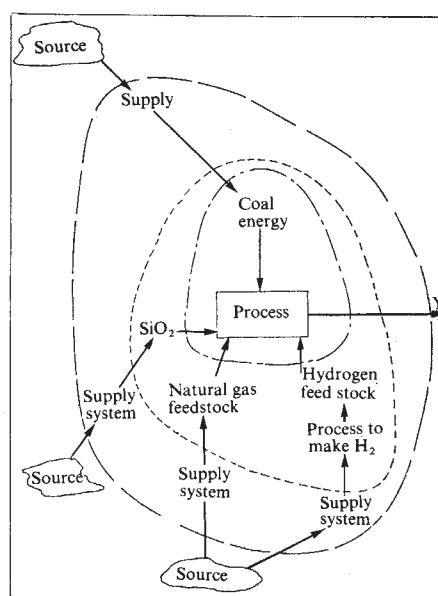


Table 1 The ERE for UK fuel industries

	1963	1968	1971-72
Coal	0.047	0.042	0.047
Gas	0.48	0.390	0.23
Oil	0.23	0.134	0.11
Electricity	3.54	3.192	2.98

Source: Chapman et al., *Energy Policy*, Sept. 1974.

This is useful in cases in which the product is itself a combustible item, such as a plastic product or a refined fuel. It takes into account the fact that the product has a future potential as a fuel. Clearly in the argument as to whether a plastic or a glass bottle is the less energy intensive, it aids the proponent of plastic bottles to take the NER, rather than the GER, while the manufacturer of the glass bottle cannot take anything but the GER.

Of all the uses of energy analysis and the use of Gross Energy Requirement, perhaps none is more spectacular than the concept of the Energy Requirement for Energy (ERE) defined as (energy of the resource sequestered—energy delivered to point of use)/energy delivered to point of use. Here we use energy analysis to assess the amount of energy that must be sequestered in order to make energy available. Table 1, for example, relates to some recent UK figures. It shows some facets of our energy industries that are not normally apparent. Thus the efficiency of fuel use for electricity generation is rapidly rising and now exceeds an average thermal efficiency of 31% at the power station. But if one allows for the energy required to get the coal and nuclear fuels to the power station, the energy dissipated in the grid and that used by the electricity boards themselves, the final ERE for electricity in the UK today is barely below 3.0. That is to say, for every unit of electricity consumed by the public (1 kWh = 3.6 MJ), some 14 MJ of energy have been sequestered.

Estimating energy requirements is not unlike cost accounting with an element of quantity surveying thrown in. One should recognise that various levels of accuracy are necessary. The direct inputs to a process under study must be estimated accurately. But the inputs to the inputs may generally be treated with a lower order of accuracy. Thus if the problem is to estimate the GER of a field of barley, one needs to know the actual inputs to the barley field. These will comprise such things as tractor fuel, nitrogen fertiliser, phosphorus, pesticide and so on. But the GER to make nitrogen fertiliser need not generally be estimated in such detail. Indeed an industry-wide aggregate based on the GER per unit of nitrogen would be sufficiently accurate. In the same way the machines to make the factories to make the inputs represent a very small part of the whole process, and may be obtained at adequate accuracy from such devices as economic input-output tables.

Perhaps the greatest room for dispute and error is in the partitioning the GER between two or more products of one process. In economics, market forces dictate partitioning. In energy

analysis, the market mechanism has yet to develop. For example, how does one partition the energy requirements in the case of the electrolysis of common salt to yield chlorine and sodium hydroxide, for each of which the current price is different? The IFIAS workshop recommended that this be done on the basis of some physical parameter, not on notions of value.

Energy analysis has been cursorily dismissed in some economic circles as being no more than "a BTU-theory of value". Energy analysts, however, would neither claim as much nor as little. The potential for energy analysis still has to be tested, just indeed as economic analysis has still to show whether it is sufficiently developed to deal with such problems as resource scarcity or a step change in the price of energy. I shall merely indicate here a few of the areas where energy analysis is being tried out and suggest that it is a logical area of endeavour.

What will be the price of ammonia fertiliser in 1990? It would be a brave man indeed who was prepared to extrapolate price trends that far ahead. Even to guess at a figure for six months from now would have its uncertainties. By using energy terms, the uncertainty largely vanishes. Figure 2 depicts how the GER for ammonia has changed with time since its first synthesis in 1912. Note how the GER appears to be flattening out at some 2.5 times the theoretical (thermodynamic) minimum. Now the thermodynamic minimum energy presupposes an infinitely slow rate of transformation—clearly not a practical state of affairs for a world that is looking for large amounts of ammonia every day. It is now clear that there is a trade-off between energy and haste of production, and a study of the new, really large (300,000 tonnes a year) ammonia plants suggest that the technological minimum at that rate of production will be not far from the value of 2.5 times the thermodynamic minimum. This simply arises from the need to have preheaters, coolers, heat exchangers and furnaces that are less than 100% efficient.

We can therefore forecast with reasonable accuracy that the GER for ammonia in 1990 will be about 45 MJ kg^{-1} .

Where less developed processes are being dealt with, of course, an assessment must be made of the effect on the GER of technological improvements and growth in the scale of operations. Economic theory would have to be used to find a means of assessing how quickly these changes might come about under the pressures of demand and supply.

Since all energy is conserved, but high quality energy is degraded to waste heat, waste has several connotations.

In one sense it is the use of more energy than necessary to effect a given transformation. Energy analysis provides the tools for looking at this problem, though it does not of itself provide the answer. Gyftopolous and associates (in *Potential fuel effectiveness in industry*, Ballinger, Cambridge, Massachusetts, 1974) estimated the Gross Free Energy Requirement (GFER) in various production processes in the USA and found that iron making, at 4.16 times the thermodynamic minimum, was much more efficient than the aluminium industry (7.6), petrol (10) or paper making (170).

But there is another sense in which energy analysis can examine waste. It

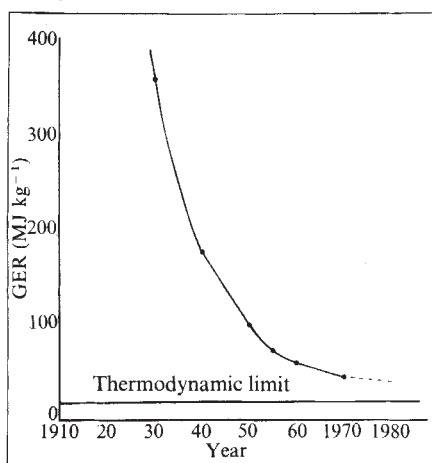


Fig 2: GER for ammonia manufacture

is now recognised that energy dissipation in highly industrialised areas can create climate modification and some climatologists are suggesting that, globally speaking, an energy dissipation equal to 1% of the incoming solar energy or even less may be an upper limit for the planet. As we approach this limit, probably in the middle of the next century, the matter of interest is not the energy dissipated by the factory in Sheffield, but the energy that had to be dissipated in order to make the Sheffield factory function. And that has to be aggregated over the whole globe. This is exactly what a GER calculation allows one to do so easily.

There are many views of the future energy situation and how the price of oil will vary. It is reasonable to argue that in intelligent hands (and OPEC is intelligent) the price of oil will be slightly lower than its substitution price. But economic substitution calculations based on pre-November 1973 energy prices are now incorrect, because the stepchange in the price of oil has raised the cost of the inputs necessary to furnish alternative forms of energy. So calculation of that substitution price is a very tricky matter. Why not re-express the problem in terms of ERE?

In 1972 it was reckoned that Colo-

rado shales would be exploitable at an energy price of \$6 a barrel, that is when oil prices reached a price of \$6 per barrel (US Energy Outlook, National Petroleum Council, USA). At that time energy cost somewhat less than \$1 a gigajoule. Allowing for others costs and profit, we deduce that the GER for shale was and is around 5 GJ per barrel. The energy in a barrel of shale oil is about 6.5 GJ per barrel. Thus shale production gives a net ERA of about 0.3. King Feisal is still sitting pretty.

Such figures are clearly very tentative and not backed by good data. But the data are obtainable.

The ceiling to energy prices will be set when the ERE for unlimited energy is known. Our likely sources are fusion and solar. At the moment the ERE for solar energy by photovoltaic methods is around 2—hopelessly high. New technology in the pipeline may reduce that by a factor of six to 0.33—better than Colorado shale—but another order of magnitude is needed before solar energy sources can compete with Middle East oil at the well head. The ERE for fusion is a matter for conjecture. In laboratory studies it is still hopelessly high.

And so, finally, we come to the logical intermediate step—to assess the GER to save energy rather to produce it. What, for example, is the GER to double glaze and insulate a house, and what is the GER saved thereby each year? When energy was cheap, economic calculations came up with different solutions. Now they are tending to come to the same solutions and people, using their own horse sense, are insulating and double glazing their homes and are finding it an extremely attractive long term gain.

One of the most telling uses of energy analysis has been the pioneer work by Chapman and Mortimer at the Open University and by Price at Friends of the Earth in their dynamic analysis of nuclear reactor building programmes. They show that no crash programme of nuclear reactor construction can ever meet an energy gap, because of the huge energy investment needed; indeed a net energy deficit would be created for the first eight to eleven years of the programme. At Strathclyde we have carried this analysis a step further using system simulation techniques to examine a range of energy scenarios for the UK to the year 2000. Though a great deal hinges on the quality of uranium ores that will be available, there seems to be no way a steam generating heavy water reactor system can sustain the rates of economic growth hoped for. Here then, is a new methodology, an old language and a familiar problem—the problem of allocation of resources. □

international news

As the current five-year plan draws to its close, considerable concern is evident in the pronouncements of the Central Committee of the Communist Party of the Soviet Union (CPSU) about the lack of progress in the Soviet research and development programme. According to *Pravda* (February 11, 1975), the plans for implementing new technological advances have not been fulfilled in "a number of enterprises of the Ministry of Power Production, the Ministry of Ferrous Metals and the Ministry of the Coal Industry. In several teams at Institutes of Higher Education, regular work on pressing problems of science and the national economy has been replaced by vain bustle between petty and inefficient themes. The possibilities of mutual exchange of achievements between enterprises, institutes and branches are not being used to the full. ... In certain institutes up to 40% of the work completed has not been realised for years". The Central Committee stresses the need for the appropriate ideological means to be taken to "put into action all reserves for accelerating scientific and technological progress", in particular, the improvement of the organisation of "socialist emulation" among the scientists and technologists concerned.

● The announcement of a new *samizdat* journal, *Twentieth Century*, edited by Roy Medvedev (the twin brother of geneticist Zhores Medvedev, who was deprived of his passport in 1973) raises once again the question of academic dissidence in the Soviet Union. Although Roy Medvedev, together with physicist Valentin Turchin, was a co-signatory of Sakharov's second "Manifesto" (1970), over the past few years there has been an increasing gap between the brands of dissidence expressed by Roy Medvedev and Sakharov. The new journal is a successor to the *samizdat Political Diary* of the late 1960s, which took, in the main, a Marxist viewpoint, although critical of the Kremlin's interpretation of Marxist-Leninist principles. It would seem that Roy Medvedev and his contributors envisage *Twentieth Century* as an alternative press for the loyal opposition—a halfway house between the official establishment journals and the more outspoken dissidence of the *Chronicle of Current Events*. The latter reports cases of breaches of constitutional

rights and the fate of prisoners of conscience, as well as discussing general issues. On the other hand, *Twentieth Century*, to judge from the contents of the first issue, seems likely to concern itself primarily, if not entirely, with theoretical debate.

Meanwhile, at the other end of the spectrum of dissidence, the appeal of Dr Mikhail Shtern against his eight-year sentence still remains postponed and unheard. His son, Avgust, who re-

Russia today

from Vera Rich



cently arrived in Israel, connects the sudden granting of his visa with his father's predicament. It is, he says, more "convenient" for the prosecuting authorities that his father should be alone, without the support of his family; it seems, in fact, that his own visa was granted on an 'either/or' basis, which left Avgust little choice but to depart for Israel. He stressed that his father is now in a very precarious state of health and that, under prison conditions, the after-effects of an old injury from a car accident could at any time produce paralysis. In his concern for his father, Avgust seemed unwilling to discuss the details of the accusation, except to state that his father has not broken or contravened any Soviet laws. He stressed, however, that Dr Shtern's chances of surviving an eight-year sentence are minimal and that only a successful outcome of the appeal can save him.

One dissident who has survived his term of imprisonment is Ilya Glezer, a neurobiologist, who was sentenced in

1972 to three years in a labour camp and three years "exile" (in Siberia). A massive appeal (151 signatures) was organised on Glezer's behalf by scientists and physicians working in related fields, asking that in the interests of international good-will he should be exiled not to Siberia but to Israel. Nothing, however, has been heard of Glezer's whereabouts since the expiration of his term of imprisonment on February 7, and it would seem therefore that the appeal has been ignored or denied.

● The Lomonosov medals for 1974 were awarded to Academician Aleksandr I. Tselikov for his work in metallurgy and metal technology, and to Angel Balevskii, President of the Bulgarian Academy of Sciences, also for his work in metallurgy. The Lomonosov medals are the highest awards of the Academy of Sciences of the USSR; two are awarded annually, one to a Soviet and one to a foreign scientist.

● The *Novosti* agency reports that a team of Leningrad scientists has developed a computer-controlled robot with laser "eyes" and a crab-like walking motion. According to the team spokesman, Mikhail Ignat'ev, it has high cross-country ability with minimum energy consumption, and is several times cheaper than a comparable air-cushion craft. It can automatically set itself to proceed at any speed from a walking pace to a "gallop", and can lift loads of up to half a ton. Ignat'ev suggests that it could be of great use in enabling geologists to cross difficult terrain—dense forests and steep mountain slopes. The team is now at work on a model which will not run down any saplings growing in its path, thereby conforming to conservation requirements.

● The February 1975 issue of the popular science journal *Priroda* carries a headline in its news section that at first glance seems an ominous echo of Lysenkoism—"Hybrid of a herb and a tree". In fact, however, the hybridisation reported is simply that of two species of *Nicotiana*—the dendroid smoking tobacco *N. glauca* and the flowering *N. alata* used in horticulture.

The research, which was carried out at the Principal Botanical Garden of the Soviet Academy of Sciences resulted first of all in sterile hybrids which were then, it is claimed, converted by "colchicinisisation, leading to doubling of the number of chromo-

somes and normalisation of the formation of sex cells" into fertile amphidiploids. It is hoped that the hybrid will form "interesting material" for the selection of disease-resistant strains of smoking tobacco—a revealing sidelight on Soviet public health planning, which, although greatly concerned with the fight against alcoholism, shows no signs of following this up with an anti-smoking campaign.

● Examination of the photographs of the Sun from the Salyut-4 mission have revealed considerable differences in the clarity of pictures taken by the same telescope, even on the same day. Although for the mission due allowance was made for the possibility of the condensation of fine particles on the optical elements, and the reflecting surface was deposited on the mirror immediately before observations were to begin, it would seem that this was not enough. In one case, one of two photographs taken only a short time apart shows a distinct image with good contrast, whereas in the other the Sun is "hardly" visible.

The Soviet space programme has always been considerably automata-oriented—the early cosmonauts being little more than human test animals in their craft. A brief note in *Pravda* on the quality of the photographs is, therefore, perhaps, of more than passing interest. After reporting that the scientists of the Crimean Astrophysical Observatory of the Soviet Academy of Sciences are now engaged in evaluating the photographs and in relating them to the spectrograms obtained with special reference to protuberances and flocculae, it wryly remarks that the scientists hope that the cosmonauts who actually operated the orbital telescope may be of "great help" in this task. □

Energy costing in the fields

by Eleanor Lawrence

THE Agricultural Research Council has recently made public the results of a Working Party on energy in agriculture which was commissioned by the relatively new Joint Consultative Organisation for Research and Development in Agriculture and Food. The report was intended to provide an appraisal of the use of energy in British agriculture and recommend ways in which agriculture could use this energy more efficiently while continuing to increase its productivity.

According to the overall energy budget calculated in the report, British agriculture at present uses only 3% of the annual national energy requirement to produce just over 50% (on the basis of cash value) of the nation's

food. Impressive as this figure is, it is difficult to find out from the report whether in fact this 3% is being used as efficiently as it might be, as ultimately the energy which makes agriculture possible comes of course from an unlimited outside source, the Sun, not taken into account in energy analysis calculations, and there are no figures given for the ways in which different levels of the inputs (fertiliser, agrochemicals and mechanisation) affect production.

Figures are given however for the proportions of the agriculture energy budget spent on petroleum, solid fuels, fertiliser and so on which can perhaps give a rough guide to where economies might begin. Petroleum accounts for 34% of the total budget, followed by fertilisers (29%) with machinery and electricity also taking a substantial share.

When figures are given for the ratios of energy (excluding solar energy) input to energy output as metabolisable food energy for various crops and animals they are on the face of it rather alarming even for some of the plants which have the supplementary input of solar energy. But the real question in trying to determine where economies are to be made is to what degree the necessary high levels of production are absolutely dependent on these massive energy inputs. Although the report does discuss this in general terms some facts and figures would have been very useful at this point.

The recommendations in fact concentrate on simple 'waste not' policies; a better use of nitrogen fertiliser, recycling of farm wastes, and some recommendations for research involving a better use of the unlimited energy sources, such as improving the photosynthetic capacity of plants and finding ways of applying biological nitrogen fixation to a wider range of crops. The recommendations often seem not to arise from the energy analysis itself but from the 'old-fashioned' experimental data discussed in the text. □

Heavenly teacher for Indian pupils

from Narender K. Sehgal, Jullundur

IF all goes well and there are no further postponements, India will in July this year embark on an ambitious experiment in mass education. At the heart of it will be a \$205 million American satellite launched in May last year from the Kennedy Space Center in Cape Canaveral, Florida. The most expensive unmanned satellite ever launched by the USA, the Applications Technology Satellite 6 (ATS 6) is designed, among other things, to help

increase literacy in India, and bring televised medical care to Alaska and special educational programmes to rural America.

According to a 1969 Indo-American agreement, the USA will let India use the 1,500 kilogram spacecraft for a year to beam educational television programmes in regional languages to selected villages in the Indian countryside. Called SITE (Satellite Instructional Television Experiment), the project is being coordinated by the All India Radio (AIR) and the Indian Space Research Organisation (ISRO), with assistance from UNESCO, the United Nations Development Programme (UNDP) and the International Telecommunication Union (ITU). Originally planned to start in 1973, SITE got delayed because of two postponements in the satellite launch. The project will now get under way in July 1975 when ATS 6 will be shifted to a position 36,000 kilometres above Kenya. From there, the satellite will receive specially prepared programmes from the Delhi and Ahmedabad stations and relay them to several thousand ground receivers in India. Out of the 6,000 villages in six states—Andhra Pradesh, Bihar, Karnataka, Madhya Pradesh, Orissa and Rajasthan—chosen for the purpose, some 2,400 will employ specially designed 'direct-reception' television sets which will pick up programmes straight from the satellite. Others, within the range of ground transmitters, will use conventional television sets.

An ISRO-built direct-reception television system was recently tested at the NASA Goddard Space Flight Center. During tests, it successfully received signals from the ATS 6. The system transmitted television test patterns from the earth station in Rosman, North Carolina, to the ATS 6.

According to officials of the AIR, by the time the satellite is moved into its appointed position, 35% of the educational programmes intended for use in SITE will have been recorded on video tape. The Earth station at Ahmedabad—modified with assistance from the ITU to transmit as well as receive television signals—will transmit to the ATS 6 these recorded programmes, which will cover education, agriculture, health and family planning. There will also be live transmissions, originating from a television studio at Ahmedabad—built with assistance from the UNESCO—which will include a 10-minute news broadcast each day. The ITU and the UNDP are also providing assistance for the installation of a low power television transmitter, as part of SITE, at Pij, a village near Nadiad in Gujarat. A total of about 1,500 hours of educational programmes will be

SWEDISH students are losing interest in research, especially in mathematics and the natural sciences. The change is part of a general movement away from full-time, examination-oriented university students straight out of school to older, part-time students who have already been employed for a number of years and who take short courses immediately relevant to their jobs and interest. The percentage of part-time students enrolling for the autumn term in Swedish tertiary educational institutions has grown continuously from 12% in 1969 to 29% in 1974.

Students used to compete for a place in a Swedish university. With increasing graduate unemployment, however, the situation began to change, and university applications have been declining since the 1968-69 academic year. This has stimulated the universities themselves to find a new type of student. Members of the new group are, on average, between 27 and 30 years old, have worked for a few years and want to study to enrich interest in the job, without necessarily taking an examination at the end of the course. A typical example is of company employees taking a short course in the economics of the firm. Of all the students who enrolled for this course in the spring term of 1974, only 15% intended to take the examinations.

The trend towards shorter courses has been institutionalised in 'distance teaching': a scheme rather like the UK's Open University except that, instead of centralising the courses under one authority, each of the six Swedish universities runs its own programme. Experiments with these courses have been running on a small scale for a couple of years. But with facilities established for the part-time student without daily access to an educational institution, the trend towards more short courses will probably continue.

All this is obviously beneficial for the new part-time students, but the general trend raises serious questions about the future of academic research. Although short courses in ecology and

environmental protection are popular, biology as such has very few takers. There is no lack of applicants for short courses in electronics, energy forms and protection against radiation, but the number of students in mathematics and

Letter from Sweden

from Wendy Barnaby, Stockholm



the natural science faculties has fallen off. Although the surplus of university graduates has been forecast to persist throughout the 1970s, it is predicted that demand from the labour market will catch up with and outgrow supply early in the 1980s. This could have serious consequences not only in the general labour market but also within the universities themselves, in maintaining the number and quality of teaching staff. And it looks as though the country's capacity for scientific research may suffer.

● How would you get rid of your old car if you lived in the countryside far from a scrapping firm, and transportation costs were high? Many Swedes faced with this problem leave cars to rot in country dumps or simply abandon them on by-roads. A recent report commissioned by the Swedish Department of Agriculture suggests measures which could be taken to stop such understandable neglect. According to the report, recirculation of metal and other wastes could save an increasing

proportion of the total energy used in Sweden.

The report foresees that recycling could save energy equivalent to 31,000 cubic metres of oil annually, or somewhat less than 1% of the country's total energy requirements. That such a small proportion is seen as justifying a reorganisation of the waste system is indicative of the current energy-consciousness in Sweden. In this affluent, packaged country, where the average household produces 270 kilograms of waste a year a person, scrutiny of energy saving measures is no longer confined to nuclear reactors, wind trappers and sun catchers—it also includes the humble kitchen bin.

The report estimates that more than 20% of household waste could be recycled. At present 70% of the average Swedish kitchen bin is filled with paper, and together these bins produce 780,000 tons of paper a year. The report recommends that householders should be made to separate out the paper from their other garbage, making it easier and cheaper for the municipalities to collect and recycle it. Chemical wastes should be recycled, dumped or deposited on a long term basis by a company jointly owned and run by the government, municipal authorities and industry. And to discourage the dumping of cars, the report proposes a co-operative system of authorisation for scrapping yards and a levy on new cars. The levy, suggested to be Skr.400 (about \$US90), would be placed on every new car sold in Sweden after July 1, 1975. When the last owner of the car wanted to dispose of it, he would take it to an authorised scrapping firm (one that met conditions for car disposal, re-sale of spare parts and so on) which would issue a receipt. On presentation of this receipt to the local authorities the owner would be refunded the levy plus a bonus of Skr.100. Since the average life of a Swedish car is 9 years, the system would pay for its own administration as well as providing subsidies to municipal authorities for cleaning up old junk-yards and abandoned cars.

broadcast during the one year experiment. Following the 1969 Indo-US agreement, the SITE Group, specially created in 1970 at Ahmedabad, was charged with the full responsibility for planning, management, operation and evaluation of the experiment. If SITE proves successful, ISRO has its sights set on a multipurpose satellite for providing nationwide television coverage and telecommunication links between Delhi, Bombay, Madras and Calcutta.

In practice, one of the most challenging tasks that SITE is likely to come up against, when the experiment actually begins, will be to keep all the

6,000-odd television receivers (the conventional ones as well as the more expensive direct-reception type) in good working order. This is going to be far more formidable a job than it might appear at first sight. The Haryana government a few years ago installed community television sets in villages within reach of the television station in bordering Delhi. But a check after a short while revealed that most of the receivers had gone wrong because the authorities had not taken steps to ensure proper care, handling, upkeep and maintenance of the sets that were installed. The SITE managers will, one

hopes, not allow this to happen.

The SITE project will in many ways be a unique venture. It will, for the first time, demonstrate that relatively inexpensive receivers (the ones built by the ISRO are said to cost about twice as much as the conventional sets) can be used to receive television programmes directly from a satellite. Moreover, such a system could open up immense possibilities and greatly enhance the chances of reducing illiteracy within a relatively short period of time. That is why, ISRO officials believe, SITE results will be of interest to many other countries. □

correspondence

Facts

SIR,—Although I am reluctant to advertise my own philosophical wares in your correspondence column, I believe that some distinctions and definitions made in my book *Scientific Knowledge and Its Social Problems* may be useful for removing some of the confusions referred to in your leader "No longer so much solid ground" (February 13).

There I constructed a sequence of stages of development of materials towards the condition of being "facts" or "knowledge"; and in this it was clear why the published "research report" is not, and cannot be, completely reliable fact. In brief, I start with the problem, and consider the essence of scientific work as the *investigation* of problems (involving creation as well as solution). Contact (necessarily partial and indirect) with the external world is made in the production of *data*, this is then refined to form *information* about the conceptual objects of the scientist's enquiry. But the problem is solved by the *conclusion* to an argument about these objects; and in this structure the information functions as *evidence*. Of course, there is a cyclic interaction between the different phases of this work; and since logical certainty is never possible, it must be governed by socially imposed criteria of *quality* and *value*. For this reason, the social mechanisms of *quality control* are essential for the progress of science; *shoddy* and *vacuous* work (easily explained in this schema) are as "natural" as good work. The *published research report* stating conclusion and evidence is therefore fallible in a number of respects.

The *social* phase of the achievement of scientific knowledge proceeds by judgements of disinterested, competent colleagues. They ask: is this report *relevant* to my enquiry, and if so is it *reliable* for use? If it passes both these tests, it enters the current stock of *tools* for future work. There, it is *transformed* in various ways, depending on the context (descendant problems, application elsewhere, or teaching). If there remains some content that is *invariant* under the inevitable changes in meaning of its terms, then we can call it a *fact*. Facts which survive the demise of the area or field in which they took rise, are then considered as very reliable facts, or bits or *scientific knowledge*.

To be brief, I have omitted all mention of the elaborations of this schema, and of its unsolved philosophical problems. But it seems to provide a reasonable schema of scientific work, and a vocabulary for describing its products.

Yours faithfully,
JEROME R. RAVETZ

London EC4

Probability

SIR,—I wish to suggest the use of a notation which I have found handy, and which seems to fulfil a real, if minor need. It is often necessary to use the phrase "the probability (of some foregoing value or result) under the null hypothesis" or, equivalently, "the probability, given H_0 ." I recommend that this phrase be rendered as $p|H_0$.

Yours faithfully,
M. HAMMERTON

University of Newcastle upon Tyne

Food and allotments

SIR,—Your correspondent F. P. Hughes of Ontario, Canada (February 27) is right in stressing the possible importance of allotments in alleviating a food shortage, but he overstates his case. Allotments in Britain certainly did not produce more than half our food during the world wars. During the 1939–45 war there were 1,500,000 allotments, covering rather less than 150,000 acres of land. There were perhaps as many plots, of a similar area, on which vegetables were grown in private gardens. This area of some 300,000 acres made a useful contribution, perhaps as much as 5%, to our food supplies. It would be difficult to exceed this proportion in most Western countries.

Yours faithfully,
KENNETH MELLANBY

Monks Wood Experimental Station,
Abbots Ripton

Teleological vs teleonomic

SIR,—Larkins, MacAuley and MacIntyre (*Nature*, November 29) suggest "a teleological explanation for the siting of the 25 HCC-1-hydroxylase enzyme system in the kidney tubule, where it is able to sense and respond to fluctuations in calcium concentration in the main outflow site from the extracellular compartment".

I take issue with the use of the word teleological in that context. Teleology is defined as the "study of evidences of design in nature", and design as "a

deliberate purposive planning". I doubt that this is the kind of explanation intended by Larkins *et al.* for the siting of an enzyme system in the kidney.

C. S. Pittendrigh in *Behaviour and Evolution*, suggested that the word teleological should be reserved for cases where the idea of the end (goal) precedes the use of the means, and the word teleonomic for cases where the ends result from means that lack design (intent)—as when adaptive traits are produced by random mutations and natural selection.

Accordingly, I suggest that the explanation offered by Larkins *et al.* is teleonomic.

Yours faithfully,
WILLIAM H. GILBERT

Colby College,
Waterville, Maine 04901

Taboo research

SIR,—Your correspondent H. Fraenkel-Conrat (March 6) makes one wonder whether after all there are not "two cultures".

Scientists should not think of themselves as the present-day equivalents of Plato's philosopher-kings, who "do good" to the rest of society whether we all will or not.

Robert Boyle wrote that "Gentlemen and scholars are apt to look upon the inquiry into manufactures as a Mechanick employment; and therefore beneath them". We do have, however, HM Factory Inspectorate, with a sound basis of rational "scientific" judgment; and on that basis, we, the whole of society, decide what kind of, for example, dusts, we shall allow in our working lives, and under what conditions and how we shall regulate our exposure to the hazards of our technological culture. By the same reasoning we should surely, the whole of us, decide what it is reasonable to do about self-replicating macromolecules.

Furthermore, Fraenkel-Conrat writes as though there were nothing else to the future of man but his material well-being; surely this is a Procrustean contraction of our attitude to and place in the cosmos—it may well have been for this reason that Prometheus was condemned by the gods to such a cruel punishment as to have his vitals continually eaten by predatory birds and then renewed?

"Man shall not live by bread alone".

Yours faithfully,
R. A. DAVIS

Surbiton, Surrey

news and views

Island biogeography and the design of wildlife preserves

from Robert M. May

ISLANDS have always fascinated ecologists. From the early days of Darwin and Wallace, to the analytical theory of island biogeography developed by MacArthur and Wilson, islands have served as ready made 'evolutionary laboratories' for the development and testing of ideas about the structure of plant and animal communities.

One major aspect of this work seeks to relate the number of species present to the area of the island (which may be a real island in the ocean, or a virtual island such as an isolated hilltop or a wildlife refuge). One rough rule, which fits a surprisingly wide variety of data, is that a tenfold decrease in area corresponds to a halving of the equilibrium number of species present.

In detail, such species-area theories have two components. The first concerns the statics, and seeks to show that there is an equilibrium number of species, S , which is appropriate to an island of given area, A (and that $S \sim A^z$, with z typically in the range 0.2 to 0.3). The second part concerns the dynamics, the way the equilibrium is maintained by a balance between extinction and immigration. One line of evidence comes from studying the increase towards equilibrium on islands where the flora and fauna have been removed either by natural catastrophe (as in the case of Krakatoa in 1883), or by experimental manipulation (as in the removal of the arthropod fauna from tiny, four square metre, islets off Florida by Simberloff and Wilson). Similarly it is revealing to monitor the initial colonisation of a newly created volcanic island such as Surtsey. A second line of attack is to study the way the balance is maintained on islands which are thought to have settled into their equilibrium state (for example, some of Lack's studies, or Diamond's study of the California islands); such work is frequently beset by problems in estimating man's effects on natural turnover rates. A third approach focuses on offshore 'land bridge' islands, which in the heyday of the last ice age some 10,000 years ago

(when the oceans were typically 100 metres lower) were part of the mainland area; as these islands have been created by the rising oceans, their rich complement of mainland species has slowly declined towards the island equilibrium value. Studies by Diamond on land bridge islands in the vicinity of New Guinea, and by Terborgh (chapter 24 in *Tropical Ecological Systems*, Ecological Studies Vol. 11; Springer-Verlag, 1975) on neotropical land bridge islands such as Trinidad and Tobago, show a very consistent pattern in their rates of relaxation towards equilibrium in the species number.

Recently several people have observed that these notions have relevance to the design of national parks and wildlife preserves.

At a qualitative level, it follows that the number of species naturally maintained at equilibrium depends on the size of the park. Many species will not cross major highways (for good reasons!), and for these species the effective area of the park is halved by bisecting it with a big road: about one sixth of all such species will be gone when the system settles to its new equilibrium. In cases where one large area is infeasible, it must be realised that several smaller areas, adding up to the same total as the single large area, are not biogeographically equivalent to it: they will tend to support a smaller species total. One way to raise the equilibrium number of species in any one such smaller park is to raise the immigration rate into it. This can be done by judicious juxtaposition of the scattered parks, and by providing corridors or stepping stones of natural habitat between them. These and other aspects of the "geometry of wildlife refuges" have been developed by Jared Diamond in an invited address at the 16th International Ornithological Congress in Canberra, 1974 (proceedings to be published), and by E. O. Wilson and E. O. Willis (in a chapter of *Ecology of Species and Communities*, to be published by Harvard University Press, 1975).

These articles represent but the beg-

innings of the subject. They ignore, among other things, epidemiological aspects of park management: many scattered parks have over a single park the advantage that not all eggs are in the one basket. Moreover species-area relations only aim at a coarse-grained first approximation to the floral and faunal dynamics. They do not incorporate such things as the spatial and temporal patterns of stable oscillation which so frequently characterise populations both in mathematical models and in the real world. The botanical evidence for a 50 year cycle in elephant irruptions in the area that is today Tsavo National Park at the foot of Mount Kilimanjaro (for example, G. Caughley, *E. Afr. Wildl. J.*, in the press) suggests that such dynamic features of natural populations can create management problems when even a very large area is enclosed.

One example of a quantitative application of these ideas is given by M. B. Usher's study of the relation between the areas of twelve nature reserves in Yorkshire and the number of species of higher plants growing in them (in *Biological Management and Conservation*; Chapman and Hall, 1973). The observations fit the relation $S \sim A^{0.21}$, with the park areas varying over more than two orders of magnitude.

A particularly nice calculation has been made by John Terborgh, in the article referred to above. For the West Indian land bridge islands, he knows the number of bird species currently to be found on a given island, and can estimate (from the neighbouring mainland species abundance) the number which were present before the island was cut off 10,000 years ago. This gradual decline in species number on the islands may be described by an equation

$$dS/dt = kS^2 \quad (1)$$

Here k is a measure of extinction rate, and Terborgh takes the overall loss rate to have an interactive form, proportional to S^2 , to account for the fact that species hasten each other's extinction. A more detailed empirical and

theoretical justification for the use of this equation was independently given by T. W. Schoener at the 16th International Ornithological Congress, mentioned above. The extinction rate k may now be calculated for each island. As expected, the k values have a systematic dependence on island area.

Now comes the payoff. The island of Barro Colorado was created with the formation of Lake Gatun in conjunction with the construction of the Panama Canal, and since 1923 it has been carefully protected as a wildlife preserve. A meticulous account of the

bird species present on the island over the past 50 years has recently been published by E. O. Willis (*Ecol. Monogr.*, **44**, 153–169; 1974). From the k -versus-area patterns of West Indian land bridge islands, an estimate of the k value for Barro Colorado island may be obtained. Equation (1) can then be used to get an independent estimate of the decline in the island's number of bird species since 1923. Terborgh's theoretical estimate of 16–17 species lost is reassuringly in accord with the actual number observed by Willis, namely 15.

Proteins which bind polyenes and react with photons

from A. J. Thomson

UNTIL a decade ago rhodopsins were the only proteins known to employ a retinyl polyene as a prosthetic chromophore. A large number of rhodopsins have been found (see Knowles, *Nature*, **253**, 394; 1975). All have the common feature of the 11-*cis* conformation of the polyene chain which in the examples investigated is bound to the protein through a protonated Schiff base linkage. The action of light is to produce the all-*trans* isomer with concomitant loss of a proton.

Three additional types of protein-polyene complexes have been discovered within the last few years, revealing an intriguing variety of functions and a satisfying economy of use of prosthetic group. The newcomers, in order of appearance, are first the yellow vitamin A transporting complex, called retinol binding protein (RBP), characterised by Kanai, Raz and Goodman (*J. clin. Invest.*, **47**, 2025; 1968). This protein stabilises and solubilises in water all the mono-*cis* and the *trans* isomers of vitamin A. No photochemical reactions of this complex have been reported. The second, retinochrome, was found by Hara and Hara (*Nature*, **206**, 1331; 1965) in various kinds of cephalopod retina, such as that of the Japanese squid. This protein absorbs maximally in the range 490 to 522 nm and contains the all-*trans* isomer of the retinyl polyene bound to the protein by a Schiff base link, probably protonated. The action of light is to release the 11-*cis* isomer of retinol. The most surprising of the newcomers, however, is bacteriorhodopsin, a purple pigment found in the outer membrane of the bacterium, *Halobacterium halobium*, when it is grown in low oxygen tension (Oesterhelt and Stoekenius, *Nature new Biol.*, **233**, 149; 1971). Containing the 13-*cis* isomer of the retinyl polyene chain linked to the

protein also by means of a protonated Schiff base, the action of light is to produce the all-*trans* isomer with the loss of a proton. The polyene chain is not detached from the protein but returns in a dark, thermal step to the protonated 13-*cis* form (Oesterhelt, Meentzen, and Schumann, *Eur. J. Biochem.*, **40**, 453; 1973; Oesterhelt and Stoekenius, *Proc. natn. Acad. Sci., U.S.A.*, **70**, 2853; 1973). The result of this rapid light-driven cycle is the transport of protons across the membrane and the accompanying production of ATP. This protein is now an important tool in the study of energy coupling driven by electrochemical gradients of the type first suggested by R. J. P. Williams (*J. Theoret. Biol.*, **1**, 1; 1961) and P. Mitchell (*Nature*, **191**, 144; 1961).

The three proteins retinochrome, bacteriorhodopsin, and rhodopsin provide an opportunity for comparative studies of the part played by a protein in directing the course of a photochemical reaction. The three light-induced reactions observed are all-*trans* $\rightarrow$ 11-*cis*, 13-*cis* $\rightarrow$ all-*trans* and 11-*cis* $\rightarrow$ all-*trans*, respectively, the last two also involving loss of a proton at some stage. Retinochrome is unique in being able to catalyse, in the presence of light, the isomerisation to the 11-*cis* form of the 9-*cis* and 13-*cis* isomers of retinol as well as the all-*trans*. The quantum efficiency of the reaction is not known in the case of retinochrome but for rhodopsin and bacteriorhodopsin the efficiencies are remarkably high, 0.65–0.7 and 0.79, respectively. This poses the interesting question of how the protein can direct a photochemical reaction so efficiently along various reaction coordinates or, indeed, in opposite directions along the same coordinate.

Catalysis of an excited state reaction must overcome the fact that non-radiative

decay by vibrational relaxation to the ground state can be very fast, $\sim 10^{11}$ – 10^{12} s $^{-1}$. In order to become efficient the required photoreaction must be faster than these competing processes. It was therefore not wholly surprising that measurements of the rise time of the earliest detectable intermediate at 560 nm after photolysis of bovine rhodopsin gave a value of less than 6 ps, the experimental resolution. This was taken as evidence that the intermediate, pre-lumirhodopsin, is the first product of the primary photoprocess (Busch, Applebury, Lamola and Rentzepis, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2802; 1972). It was however recognised by these authors that the nature of a primary photoprocess occurring within $\sim 10^{-12}$ s is severely constrained. Complete *cis*–*trans* isomerisation is unlikely to take place within this time. These workers suggested on the basis of a recent crystal structure analysis of 11-*cis* retinal (Gilardi, Karle and Karle, *Acta Cryst.*, **B28**, 2605; 1972) that the conformational change involves only a rotation of the central portion of the chain from the 11-*cis*, 12-*s-cis* form to 11-*trans*, 12-*s-cis*.

If it is generally true that unimolecular photoreactions must have a very fast primary step in order to reach high quantum efficiencies, then it is interesting to ask what are the likely primary photoreactions of other polyene-protein complexes. The overall reaction of bacteriorhodopsin involves an isomerisation step and proton loss, as does that of rhodopsin. Which process is the primary fast step? In both proteins a number of intermediates have been identified by their spectral properties either by freezing out at low temperatures or by use of fast detection techniques (Lozier and Stoekenius, *Fedn Proc.*, **33**, 1408; 1974; Slifkin and Caplan, *Nature*, **253**, 56; 1975). The technique used by Slifkin and Caplan is novel, involving the excitation of bacteriorhodopsin membrane with a modulated light source followed by phase-sensitive detection, at the modulation frequency, of changes in the optical absorption of a monitoring beam. No doubt this is a difficult experiment to perform especially with a scattering material. Resonance Raman spectroscopy has provided the first direct observation of the protonated Schiff base linkage and shows that, in bacteriorhodopsin, the native protein contains this grouping whereas the proton is not present in the link after irradiation with light to produce a species absorbing at 412 nm (Lewis, Spoonhower, Bogomolni, Lozier and Stoekenius, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4462; 1974). This result corrects an earlier conclusion, also based on Raman data (Mendelsohn, *Nature*, **243**, 22; 1973). The same technique has also been used to show that an early intermediate of rhodopsin, pre-lumirhodopsin, is

protonated (Oseroff and Callendar, *Biochemistry*, **13**, 4243; 1974).

The difficult question to resolve is which species are primary photoproducts leading to a biochemical event and which competing by-products. Since the quantum efficiencies of formation of these species are not known and since the quantum efficiencies of the photo-biological processes of rhodopsin and bacteriorhodopsin are less than unity, this question remains open. In view of the function of bacteriorhodopsin it is worth examining the hypothesis that the loss of a proton from the excited Schiff base may be the primary photoprocess. Involving the motion of a proton along the N-H vibrational coordinate this transfer will be exceedingly fast, within the time of a single vibration. All that is required is a change in the basicity of the N atom in the excited state. If this were followed by a slower thermal isomerism of the polyene chain the role of the protein in selecting the course of the isomerism becomes more comprehensible. One possible test of this hypothesis might be made by measuring the quantum efficiencies of the photoprocesses of rhodopsin and bacteriorhodopsin after deuteration of the Schiff base link. A pronounced isotope effect should be seen only if proton transfer is involved in the primary photoprocess.

Globin synthesis: rates and ratios

from Pamela Hamlyn

It has been known for several years that the α and β globin chains of mammalian haemoglobin are synthesised on different polysomes and yet their production is controlled in such a way that nearly equal amounts of each are produced. The means by which these two independent events are synchronised is the subject of some interest for it seems possible that this mechanism may be one of general importance in the control of synthesis of proteins composed of two or more subunits.

Lodish and his co-workers have made several contributions to the study of globin synthesis control and recently Lodish (*Nature*, **251**, 385; 1975) has derived a simple kinetic rate equation for initiation and elongation of polypeptide chains which, although based on α and β globin synthesis, may be applicable to eukaryotes in general. A result of these calculations is the prediction that any reduction in the rate of polypeptide chain initiation will lead to preferential inhibition of translation of mRNAs with lower rate

constants for polypeptide initiation. Lodish has argued that α mRNA has a lower initiation constant than β mRNA. Based on the observation that α mRNA is found in polysomes averaging three ribosomes, whereas β mRNA polysomes have five ribosomes on average, he contends that since the rate of elongation is the same for α and β chains then α mRNA must initiate protein synthesis only 60% as often as β mRNA. (He suggests that balanced synthesis of the two globins is achieved by a 1.7-fold excess of α mRNA over β mRNA.) Globin synthesis, therefore, provides an experimental situation to test the predictions of his model. Accordingly Lodish has added inhibitors of protein synthesis initiation to a reticulocyte lysate and measured the effect on the ratio of α and β globin synthesised. In all cases he found that it was α mRNA translation that was preferentially inhibited as predicted by his model.

McKeehan, working at the Basel Institute for Immunology, has reported the results of a systematic study of globin synthesis in the reticulocyte lysate in which each of the components of the synthesis was varied in turn—keeping the others constant—and the effect on the α/β globin ratio measured (*J. biol. Chem.*, **249**, 6517; 1974). He found that increasing the concentration of globin mRNA resulted in a decrease in the α/β globin ratio, whereas increasing the concentration of 'initiation factors' (obtained from a salt wash of the small ribosomal subunit), or the concentration of the ribosomal subunits, resulted in an increase in the α/β globin ratio. When experiments were performed with separated subunits it was found that increasing the 40S subunit relative to the 60S gave an increase in the α/β globin ratio, whereas when the 60S subunit was in excess then there was a small decrease in the α/β globin ratio.

McKeehan maintains that these results can best be explained using Lodish's assumptions, namely that β globin mRNA initiates the synthesis of protein more efficiently than α mRNA, and also that there is more active α mRNA than β mRNA bound to polysomes.

Consider for example when the concentration of 40S ribosomal subunit is increased, all other components being held constant. The excess of small subunit means that α and β mRNA are no longer in competition for initiation so that the superior efficiency of β mRNA initiation is not involved in determining the α/β globin ratio produced in the reticulocyte lysate. Only the relative concentrations of the two messengers is effective in determining the globin ratio and since there is more α mRNA then the α/β globin ratio

increases, as is observed experimentally.

Both Lodish and McKeehan make the point that these mechanisms for altering the ratio of α/β globin synthesised rely only on varying the relative amounts of the components of protein synthesis and do not require existence of α and β mRNA specific initiation factors.

The results and predictions of Lodish and McKeehan go some way to defining the components in the mechanism of control of globin synthesis but do not explain how the feedback could operate *in vivo*. In other words, adding more 9S RNA to the constituents of protein synthesis may increase the relative amount of β globin but how is the lack of β globin communicated so that more 9S mRNA is made available to the ribosomes?

rosy structural gene for xanthine dehydrogenase

from Benjamin Lewin

WHAT is a gene? This question has been resurrected by the present debate on the nature of the unit of gene expression in eukaryotes. At the heart of the discussion is the apparent discrepancy between the coding potential of the eukaryotic genome and its probable number of genes. This can be placed on a satisfactory quantitative basis really only with *Drosophila*, where a visible division of the genetic material into about 5,000 bands is evident in the polytene chromosomes of the salivary glands. That each band represents a single genetic function is suggested by the identification of individual lethal complementation groups with single bands in a region of the X chromosome (Judd, Shen and Kaufman, *Genetics*, **71**, 139–156; 1972) and by the rough equality between the total number of sex-linked lethals and X chromosome bands (Hochman, *Cold Spring Harb. Symp. quant. Biol.*, **38**, 581–598; 1973) and between the total number of third chromosome late lethals and bands (Shearn, *Genetics*, **77**, 115–126; 1974). But the average content of a band is about 30,000 base pairs, sufficient to code for 10–20 proteins of average size. Two extreme views can be taken of this situation: all genes may be identifiable by lethal mutations, so that each band contains only one protein-coding sequence, on average occupying about 10% of the DNA; or only a small minority of genes can be identified by lethal mutation, so that there may be many 'null loci' that are unidentifiable by mutation, in which case there might

be some 50,000 genes with many in each band (see Lewin, *Nature*, **251**, 373–375; 1974).

Another approach to defining the genetic unit is to determine the nature of mutations at some locus. The mutations mapping in one complementation group may include *cis*-dominant control elements as well as structural genes; since most loci are identified by the effects which mutations in them have upon the phenotype, there

is usually no way to tell whether they represent alterations in a control element or structural gene. To determine the class of mutational event therefore demands analysis of loci where a phenotypic effect can be recognised and the protein product is known.

rosy mutants in *Drosophila* have eyes that are brownish in colour (instead of the wild type red colour). Two sets of observations suggest that this locus is connected with the enzyme xanthine

dehydrogenase. A dosage effect exists in which heterozygotes with only one *ry*⁺ gene possess half of the wild type level of enzyme activity; and abnormal flies with three *ry*⁺ genes have 150% of the wild type level (Grell, Z. *Vererb.*, **93**, 371–377; 1962). This could mean that *rosy* mutations act in a control element to prevent synthesis of XDH or that they represent alterations in the structural gene completely preventing enzyme activity. Isoalleles that affect the electrophoretic mobility of XDH (but which are wild type in eye colour) map close to the *rosy* mutations, an indication that the structural gene is in or close to the *rosy* locus (Yen and Glassman, *Genetics*, **52**, 977–981; 1965). By fine mapping of some of these isoalleles, Gelbert, McCarron, Pandey and Chovnick (*Genetics*, **78**, 869–886; 1974) now define their relationship to *rosy* mutations in a report on the organisation of this locus.

rosy mutants entirely lack XDH activity, since even low levels of this enzyme are sufficient to allow expression of the *ry*⁺ phenotype, so that it is the *ry*⁺ isoalleles alone that can be used to define the structural gene by their effect on the mobility of the enzyme. Since it is not practical to map such sites directly, the first step in this analysis was to derive *rosy* mutants from each of five of the *ry*⁺ isoalleles. When two such *rosy* mutants are crossed, *ry*⁺ progeny can be selected by growth on a purine-supplemented medium which kills larvae lacking XDH. In these experiments, 170 *ry*⁺ survivors were obtained from a total of 7×10^6 zygotes. The markers present on either side of the *rosy* locus were used to determine whether a crossover or gene conversion event was responsible for generating the *ry*⁺ progeny. Examination of these strains to determine whether they have the XDH electrophoretic mobility characteristic of the maternal or paternal parent can then be used to map the site responsible for the electrophoretic variation. In effect, the *ry* mutant sites are selected markers in these crosses and the sites responsible for electrophoretic mobility comprise unselected markers.

The electrophoretic (*e*) sites mapped in two clusters, one at each end of the region identified by *rosy* mutations. This leaves open the possibility that the structural information for XDH might be located in two elements separated by some distance. By using a *cis/trans* test, however, Gelbert *et al.* demonstrated that *e* sites at both extremes appear to fall within the same protein-coding element. This is consistent with the observation that the enzyme XDH consists of a dimer of two identical subunits of about 130,000 daltons; this should require a coding length of a little more than 3,000 base

Strong interactions of the ψ

from David J. Miller

SINCE the ψ (or J) particles were first discovered at Brookhaven and the Stanford Linear Accelerator Centre in November 1974 (see *Nature*, **252**, 438; 1974 and *Phys. Rev. Lett.*, **33**, 1404, 1406 and 1408; 1974), experimental physicists all over the world have been looking for their effects. Some have scanned the data already collected from previous experiments, others have made proposals for new and more refined experiments, but much of the data now appearing is coming from apparatus which just happens to be set up at the accelerators or storage rings, and which has been rapidly adapted to look for the new particles.

The world of physics has been swept by waves of gossip, often inaccurate, and it has become necessary to separate 'mezzofacts' from real facts which can be reliably traced back to their authors.

The latest pieces of information to leave the mezzo-fact category are data on the photoproduction of the ψ (or J) with a mass of $3.1 \text{ GeV}/c^2$. The Brookhaven experiment showed that this ψ is produced with a very low probability in proton-proton collisions. The SLAC, and other subsequent e^+e^- experiments, saw copious production. These two results implied that the ψ takes part in electromagnetic interactions, but might not have strong interactions with nucleons (protons or neutrons). In photoproduction, a particle is produced by bombarding a nuclear target with high-energy γ rays. If the ψ is a 'vector' particle, like the ϕ meson (as discussed in *Nature*, **252**, 438; 1974), then an interacting photon could sometimes behave as if it were an incoming ψ . The photoproduction of the ϕ , and the related ρ and ω vector mesons, are very well explained by treating the incoming photon as a vector meson, which then has a strong interaction with the target nucleon. This is called the 'vector meson dominance model'

(VMD) of photon-hadron couplings. If the ψ is a hadron (a strongly interacting particle) then the model would predict the rate of ψ photoproduction, with only two important parameters: the strength of the coupling of γ to ψ , which is measured directly by the $e^+e^- \rightarrow \psi$ experiments, and the cross section (a direct measure of probability) for ψ -nucleon scattering.

Three groups have produced results in searches for ψ photoproduction. At the Cornell electron synchrotron (*Phys. Rev. Lett.*, **34**, 231; 1975) no sign of ψ production has been seen with 11 GeV photons. An experiment at the SLAC linear accelerator has also seen no ψ production from 18 GeV photons (*Phys. Rev. Lett.*, **34**, 288; 1975). But news has come from the Fermi National Accelerator Laboratory near Chicago that a group led by Professor Wonyong Lee of Columbia University has seen ψ photoproduction by photons of about 100 GeV produced from the 400 GeV proton-synchrotron. This result was a mezzo-fact for some months, but has now been reported by Lee himself in an FNAL seminar and can be regarded as authentic.

The tentative rate for ψ photoproduction at FNAL appears consistent with the limits put upon it by the two experiments which saw no effect. Using the VMD model it is clear that Lee's result implies a cross section for ψ -nucleon collisions somewhere in the region of one millibarn (10^{-28} cm^2). Only the strong interaction could give such a large cross section, but the cross sections of other strongly interacting particles are all in the region of 20 to 40 millibarns. The ψ therefore seems to be a 'semi-strong' particle. It is likely that this inhibited interaction probability is linked to the narrow width of the ψ and slowness of its decays, but there is no agreement on what the link may be.

pairs. This length fits well with the genetic analysis of *rosy*, since *ry* mutants span 9×10^{-3} recombination units, and 0.01 units has been estimated to represent almost 4×10^3 base pairs. The *rosy* locus lies in a region of five bands on the polytene third chromosome and since these are of average size this implies that whichever band contains *rosy* has about ten times more DNA than codes for the protein. The *rosy* site, lying at map position 52.0, is bounded by *kar* at position 51.7 on one side and by *1(3)26* at position 52.2 on the other and the isolation of further mutations may, of course, permit a more exact resolution of its extent.

If it is true that the distribution of mutant sites within control elements and structural genes directly reflects their relative sizes, these results show that the *rosy* locus represents a structural gene; for if only part of the region identified by *rosy* mutations had the function of coding for protein, the sites responsible for electrophoretic variation would lie in one small cluster instead of extending to both ends of the genetic map. In support of this contention, Gelbart *et al.* argue that the *rosy* mutations which they selected by X-ray mutagenesis largely represent single base pair alterations. Point mutations in this case must be able to abolish completely the activity of XDH. By using mutagens inducing other types of changes that perhaps might be prone to have effects on control elements, in the future it may be possible to identify regulatory *rosy* mutations. The conclusions that the *rosy* locus is largely occupied by the structural gene for XDH depends also upon the assumption that recombination rates are constant per unit length of DNA throughout both control element and structural gene; for if recombination takes place at a greater frequency in the structural gene than in the control element, the apparent size of the structural gene would be exaggerated relative to the control element. The failure of Chovnick and his colleagues to observe unequal crossing over in their earlier extensive analysis of recombination at the *rosy* locus argues that the structural gene itself comprises nonrepetitive DNA, but leaves open the possibility (now under study) that adjacent control elements might represent repetitive sequences in which recombination is suppressed in order to limit such events. The most probable explanation of these results therefore seems that the *rosy* locus represents the structural gene for XDH, occupying only some 10% of the band in which it is located. Whether the remaining DNA represents control functions associated with the XDH structural gene and thus defines a single genetic unit remains a point for future research.

Ring galaxies and intergalactic gas clouds

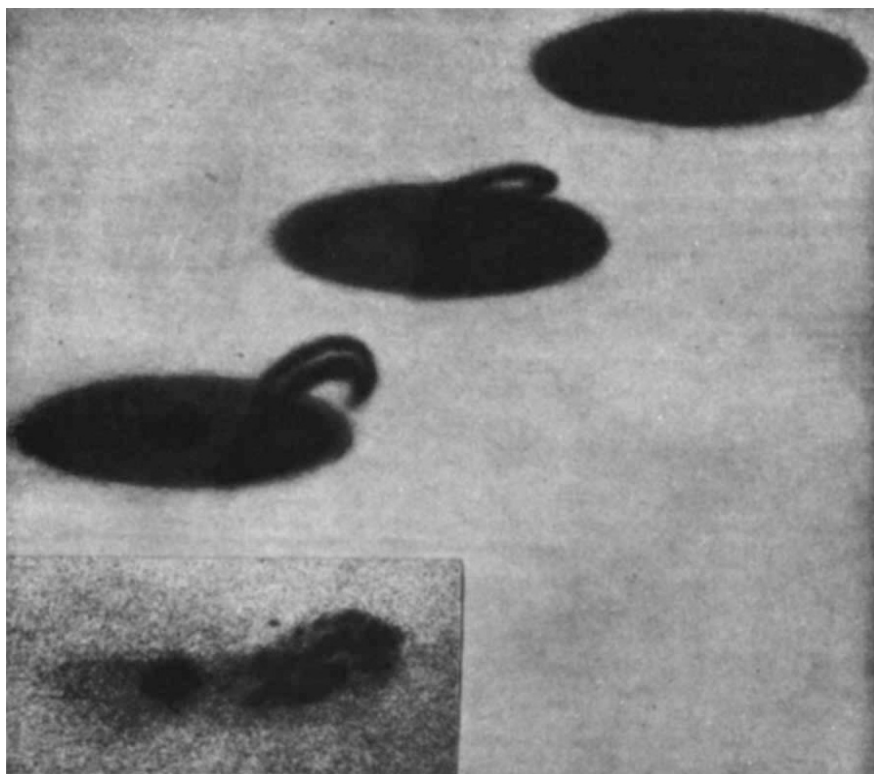
from Craig D. Mackay

In a recent article in *The Astrophysical Journal* (194, 569; 1974), Freeman and de Vaucouleurs attempt to account for the formation of two types of object usually dismissed as "irregular galaxies". The first appears as an irregular ring of gas with a number of condensations within it, the second (an example is shown at the bottom of the accompanying figure) appears as a spheroidal galaxy composed almost exclusively of stars with a small region filled with several blobs of gas.

The ring galaxies have a number of features similar to those of the annulus of gas found in many spiral galaxies. Freeman and de Vaucouleurs consider the possibility that such an annulus was stripped out of a normal spiral galaxy to form a ring galaxy by a collision with an intergalactic gas cloud. The presence of large clouds of neutral or ionised gas moving within clusters of galaxies has been suggested as a convenient way of stabilising clusters of galaxies. The scenario proposed is as follows: Suppose that a gas cloud collides with a normal spiral galaxy as is shown in the artist's impression in the upper part of the figure. Stars, being small and massive, are not affected by the colliding gas cloud. The annulus of gas, however, is

progressively stripped out of the galaxy as shown in the figure. Some of this gas may be attracted back towards the massive disk of the galaxy, giving rise to the 'hook' in the lowest of the artist's sketches, a sketch which looks remarkably like the object NGC 7828-29 shown at the bottom of the figure. Eventually all the gas is swept out of the galaxy and a spinning ring of gas is formed moving away from the parent galaxy. This gas ring is unstable and expands (because of the unbalanced centrifugal force) until it is braked by the background gas in the cluster. On fairly short time scales ($\sim 10^8$ years) instabilities in the gas cause knots to condense in the ring giving structures very similar to the ring galaxies observed.

The main difficulty with Freeman's and de Vaucouleurs' model is that it is important the collision happens rapidly enough to ensure that all the gas is swept out in a small fraction of the rotation time of the galaxy (otherwise the ring structure will become grossly distorted). A second problem is that it is difficult to avoid heating the gas ring in such a collision to an extent that the ring structure is completely disrupted. There is, however, no doubt that their model gives a compelling account of the formation of these hitherto unexplained galaxies. Indeed, the elegance of their model ensures that it will receive further serious attention and may help to explain a number of the many other unusual galaxies which are all around us.



Artist's conception (above) of three typical phases of penetration of disk galaxy passing through an intergalactic cloud, with progressive separation of gas annulus from disk; (bottom) photograph of NGC 7828-29.

Seismic activity before earthquakes

from Peter J. Smith

It is now well known that many moderate and large earthquakes are immediately preceded by smaller foreshocks; that is, over periods ranging from weeks to hours before significant seismic events there is an increase in seismic activity. But what happens to the seismicity in the vicinity of an earthquake epicentre during the preceding months and years? Here opinions are more divided, with some studies concluding that the level of regional seismicity also generally increases over the longer time scale and others suggesting that earthquakes are frequently heralded by a period of relative quiescence.

Mogi (*Bull. Earthquake Res. Inst.*, **47**, 395; 1969), for example, concluded from a study of four great Japanese earthquakes that whereas the immediate epicentral area becomes relatively calm for some years prior to the main shock, activity in the surrounding regions increases markedly. He likened the pattern of seismicity to the shape of a ring doughnut centred on the epicentre. A somewhat similar pattern was also observed by Borovik *et al.* (*Izv. Acad. Sci. USSR, Phys. Solid Earth*, **2**, 21; 1971) in a quite different tectonic setting. Borovik and his colleagues found that the area surrounding the 1959 Baykal (Soviet Union) earthquake was relatively quiet seismically for several years before the event and that the quiet area, or 'preparation region', enclosed the aftershock zone. During the 7½ years preceding the main earthquake, only two (smaller) earthquakes were observed within the preparation region whereas 20 were recorded in an outer region of comparable area. After examining the geological structure and seismicity of southern California, Allen *et al.* (*Bull. Seismol. Soc. Amer.*, **55**, 753; 1965) even went so far as to propose that areas of seismic quiescence may indicate the sites of future large earthquakes.

Fedotov *et al.* (in *Earthquakes and the Deep Structure of the South Kurile Island Arc*, Moscow, 1969) have taken precisely the opposite view, arguing from their study of the Kamchatka-Kurile-Japan seismic zones that impending great earthquakes are heralded by an increase in seismic activity beginning 5–20 years before. In this they are supported by Sadovalsky *et al.* (*Tectonophysics*, **14**, 295; 1972) who suggested on the basis of Asian studies that an increase in seismic activity over a 5–10 year period indicates a forthcoming strong earthquake. Suyehiro and Sekiya (*Tectonophysics*, **14**, 219;

1972) also found that the seismicity in the vicinity of the great Kanto earthquake of 1923 was significantly greater during the few years before the shock than over the period 1926–1972. And finally, Tocher (in *San Francisco Earthquakes of March 1957*, California Division of Mines and Geology, Spec. Rep. 57, 1959) concluded that for several decades preceding the Hayward earthquake of 1868 and the San Francisco earthquake of 1906 moderate earthquakes probably occurred at a greater frequency than they do on average over the longer term. He thus interpreted increasing regional seismicity as a symptom of a strain buildup which could subsequently lead to a major shock.

So which side is right? Or is the conflict more apparent than real, either because different earthquakes are associated with genuinely different precursory phenomena or because experimental and analytical techniques have hitherto been imperfect? In an attempt to resolve the matter, Kelleher and Savino (*J. Geophys. Res.*, **80**, 260; 1975) have now carried out an extensive study of the patterns of seismic activity preceding large strike-slip and thrust-type earthquakes which have occurred along the northern, north-western and eastern margins of the Pacific. The events include the San Francisco earthquake of 1906, the Kamchatka event of 1952, the Chile shock of 1960, the great Alaska earthquake of 1964 and several others, most of which had rupture zones extending hundreds of kilometres. In each case the foci of all shallow (<100 km) events preceding the main shocks were relocated, in some cases for precursory intervals of up to 45 years.

The observed patterns of seismicity preceding large earthquakes were found to correlate with the configurations of the shocks' rupture zones and with the positions of the main event epicentres within the zones. Specifically, extensive parts of the rupture zones were relatively aseismic until the times of the main shocks, and the low levels of seismicity applied to events at least several magnitudes smaller than the main shocks and probably to events many magnitudes smaller. On the face of it, this would seem to support those arguing for precursory quiescence; in practice, the detailed picture looks rather different. In most of the earthquakes examined by Kelleher and Savino rupture began in an area of moderate seismic activity, propagated up to hundreds of kilometres into adjacent quiet regions, and in many cases ended in areas with marked prior seismicity. Thus prior seismic activity often occurred near the epicentres of the main events and near the edges of the rupture zones but not throughout

the main bodies of the zones.

These general principles are well illustrated by the Kamchatka earthquake of 6 November 1952. During the earthquake, rupture propagated from the focus towards the south-west, parallel to the strike of the thrust plane and throughout an elongated zone 400–500 km long and 100–200 km wide. For 30 years before the earthquake, most of the rupture zone was relatively quiet seismically; but throughout this period there was a clustering of activity around the position of the subsequent epicentre at the north-east of the rupture zone and around the opposite (south-western) end of the zone. The bulk of the rupture zone only became seismically active with the aftershocks of the main earthquake.

There is some indication from the Kamchatka and other large earthquakes that, where prior activity does occur (especially in the epicentral regions), its level increases as the main shock approaches. On the one hand, there is no suggestion that precursory seismic activity appears in the gaps where none occurred before. Gaps in seismicity for great earthquakes along major plate boundaries seem to be gaps for smaller magnitude activity also; and these gaps remain until and unless they become aftershock zones. Unfortunately, the available data are insufficient to enable temporal variations to be determined in any great detail. Thus it is not yet possible to say whether a seismic gap becomes quiescent as part of a definite process leading to a large earthquake or whether, once the aftershocks of a large event have died away, the gap remains quiescent until the next large shock. At present there are data to support either behaviour but not enough to decide which predominates. What the new study by Kelleher and Savino has made clear, however, is that the spatial distribution of seismicity is critical in any attempt to describe temporal variations. It is not enough to say that a particular earthquake was preceded by relative quiescence or a regional increase in the level of seismic activity. The precise points at which these phenomena do or do not take place must be clearly determined.

Turnover of motor endplate

from Angela Vincent

THE transmission of impulses at the neuromuscular junction is achieved by the release of acetylcholine from the nerve ending. Acetylcholine interacts with receptors on the endplate of the muscle and causes a change in sodium and potassium permeability which

results in propagation of the impulse along the muscle fibres and activation of the contractile apparatus. In mammalian skeletal muscle the endplate has a diameter of 20–60 μm , but its surface area is increased by infolding of the postsynaptic membrane. The acetylcholine receptor in normal muscle is confined almost exclusively to the endplate, as has been shown autoradiographically by labelling with the specific and irreversible blocking agent α -bungarotoxin (α -Bgt) (Lee, Tseng and Chiu, *Nature*, **215**, 1177; 1967; Barnard *et al.*, *Nature*, **234**, 207; 1971). Recent estimates of receptor sites suggest a density of $2\text{--}4 \times 10^4 \mu\text{m}^{-2}$ (Fertuck and Salpeter, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1376; 1974; Porter and Barnard, *J. Membrane Biol.*, **20**, 31; 1975). When the nerve to a muscle is cut, acetylcholine sensitivity begins to appear over the entire surface of the muscle fibre and reaches a maximum in 2–3 weeks, only declining as reinnervation takes place (Axelsson and Thesleff, *J. Physiol., Lond.*, **147**, 178; 1959; Miledi, *J. Physiol., Lond.*, **151**, 241; 1960). Radioactively labelled α -Bgt binding again follows this closely: binding increases 20-fold overall (Miledi and Potter, *Nature*, **233**, 599; 1971), but the density of extrajunctional sites, at peak $600 \mu\text{m}^{-2}$ (Fambrough, *J. gen. Physiol.*, **64**, 468; 1974), never approaches that at the endplate itself. Despite the translation freedom found for many membrane-bound proteins the normal endplate receptors remain discretely localised—even after denervation and degeneration of the nerve ending.

What makes the endplate so stable? Berg and Hall (*Science*, **184**, 473; 1974) labelled rat denervated diaphragm with ^{125}I - α -Bgt *in vivo*, removed the muscles and cultured them for 24 hours. They found that the extrajunctional radioactivity declined with a half life of 8 h, whereas there was only a marginal reduction in endplate labelling during that time. The rate of ^{125}I loss was reduced considerably by metabolic or protein synthesis inhibitors, indicating that little of it was due to reversibility of the toxin–receptor complex. Radioactivity recovered from the medium was mostly degraded toxin, although native toxin added to the culture was not broken down. They concluded that the extrajunctional toxin–receptor complex was being metabolised considerably faster than the endplate complex. Chang and Huang (*Nature*, **253**, 643; 1975) confirmed these findings, but they left the ^3H - α -Bgt-labelled diaphragms *in situ* for several days before removing them. They found that the turnover of endplate receptors had a half life of about 7.5 days which could be shortened by conditions which are known to increase the synthesis of new receptor, such as denervation per-

formed at the same time as labelling. Their value for the half life of extrajunctional receptors in rats denervated 9 days previously was 19 hours, somewhat longer than Berg and Hall found in rats which had been denervated 5 days previously.

Does this different turnover reflect differences in the receptor molecules themselves, or in their mode of incorporation into the membrane? Several reports suggest that the two classes of receptor in intact muscle are not identical—with respect to their sensitivity to cholinergic antagonists (Bernanek and Vyskocil, *J. Physiol., Lond.*, **188**, 53; 1967 and Lapa *et al.*, *Exp. Neurol.*, **43**, 375; 1974) and their acetyl-

choline noise spectra (Katz and Miledi, *J. Physiol., Lond.*, **224**, 665; 1972). On the other hand, using detergent-solubilised receptors, Chiu *et al.* (*Biochem. Biophys. Res. Commun.*, **51**, 205; 1973) showed that both receptors had the same apparent molecular weight on gel filtration; and Alper *et al.* (*FEBS Lett.* **48**, 130; 1974) found very similar 'protection constants' for several cholinergic ligands with respect to α -Bgt binding.

Many of the α -Bgt binding studies have been done on the intact rat diaphragm but this has certain disadvantages since one is dealing with a slowly diffusing polypeptide which makes kinetic analysis difficult. In *Biochemistry* (**13**, 5522; 1974), Almon, Andrew and Appel report some interesting results with Triton-solubilised receptors from rat leg muscles. They show ^{125}I - α -Bgt binding isotherms for normal and denervation-induced receptors. In both cases they find that the binding follows that predicted for a homogenous ligand interacting with a single class of independent sites. They find, however, that the toxin has a 10-fold higher affinity constant in denervated muscle extracts. More important, prolonged exposure to 1% Triton X-100 changes the toxin's affinity for the normal receptors to that found for the extrajunctional ones, although the number of sites remains the same.

Until now, the separate purification of the two types of muscle receptor has seemed a formidable task, because of the very low yield obtainable from normal muscle. Nevertheless, in the Abstracts of the Biophysical Society (*Biophys. J.*, **15**, 332a; 1975) Brookes and Hall report the purification, by affinity chromatography, of normal and denervation-induced receptors from rat diaphragm. Apparently, the two receptors are both glycoproteins and indistinguishable on gel-filtration and zone centrifugation. But they do exhibit some differences in affinity for α -Bgt and sensitivity to D-tubocurarine, and show separate peaks on isoelectric focusing.

At present it looks as if the normal and denervation-induced receptors are not very different, at least in macromolecular terms. The very high density of receptors on the postsynaptic endplate membrane makes interactions between them seem a possible explanation for the lower rate of turnover and other differences observed *in situ*. There is, however, no evidence that the solubilised endplate receptors are aggregates of the less densely packed extrajunctional ones. On the contrary, the results of Almon *et al.* suggest that other hydrophobic components could interact with the receptors in the membrane-bound state, and survive to some extent in mixed Triton micelles on



A hundred years ago

The third paper was by Mr B. Tower, on a method of obtaining motive power from wave motion. He said that this inquiry originated with Mr Deverell, who came home from the antipodes for the purpose of promulgating it. Mr Deverell's proposition was to suspend a heavy weight on board a ship by means of springs, and to obtain motive power by the oscillation of this weight through a distance not more than the height of the waves. It however appeared to Mr Tower that since the centrifugal force of wave motion in a vertical direction is alternately added to and subtracted from, the force of gravity thereby causing a virtual variation of the intensity of that force, the question might be broadly stated as follows:—

Supposing the force of gravity to vary in intensity at regular intervals, that is, to become alternately greater and less than its normal amount, what is the best means to obtain the maximum amount of energy from a given weight oscillating under the influence of these variations?

... Now, as energy or power is defined as force moving through distance, it is clear that the quantity of energy or power to be obtained by this system will depend on the distance through which this weight is caused to move during each successive variation of gravity. . .

The first experiments Mr Tower made with a model apparatus constructed on these principles showed him that the best arrangement would be to put a weight on the end of a revolving arm, whereby the centrifugal force of the wave motion might be utilised as well as the rising and falling motion.

from *Nature*, **11**, 410; March 25, 1875.

solubilisation. In this context it is interesting that Fambrough (*Neurochemistry of Cholinergic Receptors*, edit. by E. de Robertis, Raven Press, New York) observed that clusters of toxin-labelled receptors on cultured chick muscle cells could be dispersed by 0.01% Triton, although this treatment did not solubilise the receptors or irreversibly damage the membrane.

It seems that to establish the membrane characteristics which are responsible for the stability of the end-plate it will be necessary not only to investigate the structure of the receptor protein itself, but also to look more closely at the involvement of other membrane components in the intact tissue.

Conservation of nitrogen in climax ecosystems

from Peter D. Moore

CLIMAX ecosystems possess several attributes which lead to an increased conservation of essential nutrients; for example, nutrients generally become concentrated in the biota, and nutrient output from the system is low in comparison to this biotic reservoir (Bormann, Likens and Eaton, *Bioscience*, **19**, 600; 1969). In a series of papers, Rice and Pancholy (*Am. J. Bot.*, **59**, 1033; 1972 and **60**, 691; 1973) have now suggested that certain climax vegetation types may inhibit nitrification by soil microbes, as a result of the production of toxins in the litter, thus reducing the rate of nitrate production and loss.

In the process of nitrification ammonium ions are oxidised by microbes in two stages to nitrite and then to nitrate, in which form nitrogen is more readily available to the majority of plants. In this form, however, nitrogen is easily lost from ecosystems since, being anionic, it is not retained well by clay colloids in the soil. Rice and Pancholy therefore regard the suppression of nitrification as a mechanism whereby nitrogen is retained within the ecosystem.

They were led to this position initially by a number of reports concerning grassland habitats in which low activities of the nitrifying bacteria and very low nitrate levels were found. Theron (*J. Agric. Sci., Camb.*, **41**, 289; 1951) had suggested that grass roots could secrete a toxin which might affect nitrification and, in African grasslands, Boughey *et al.* (*Nature*, **203**, 1302; 1964) suggested that the secretion of toxic substances by the grass *Hyparrhenia* could explain many aspects of the ecology of these habitats. Rice and

Pancholy followed up these ideas in an old field succession in Oklahoma by measuring ammonium and nitrate levels in soils from various stages in the succession.

They showed high nitrate levels in the early stages falling to low levels at the climax, with ammonium ions behaving in a reciprocal fashion. They also found that the density of *Nitrosomonas* and *Nitrobacter* (the nitrifying bacteria) in the soil fell during the course of succession. In 1973 they published further data implicating tannins and their derivatives as possible agents in the suppression of nitrification and showed that tannin levels were highest in the climax soils. Particularly surprising were the high tannin concentrations in grassland soils and in the litter produced by grasses. This, they felt, could account for the low nitrate levels found in many grasslands, particularly since *Nitrosomonas* activity is inhibited by tannin levels of only 2 p.p.m., less than half the lowest recorded soil concentration in grasslands. *Nitrobacter* (which oxidised nitrite to nitrate) was less sensitive, but suppression of one step would effectively block nitrification.

Recently (*Am. J. Bot.*, **61**, 1095; 1974) they have attempted the separation and identification of the precise inhibitors which may be involved and, by analysing acetone extracts of plants and soil, have come to the conclusion that many compounds in addition to tannins could be active in suppression, mainly phenolic acids and phenolic glycosides. All were more effective in suppressing *Nitrosomonas* than in the inhibition of *Nitrobacter*.

Meanwhile, back in the African grasslands, more work has been in progress on the suggested inhibitory properties of *Hyparrhenia*; Purchase (*Plant Soil*, **41**, 527; 1974) has added the washings of this grass to soils and has checked the progress of nitrification. After 13 days nitrate production began to fall, but Purchase regards this as the result of increased acidity, since addition of CaCO_3 to the water led to continued nitrate production. Eluate of the soils seemed not to influence the activity of test *Nitrobacter* cultures, but Rice and Pancholy have shown that the nitrite to nitrate stage of the oxidation is less sensitive to inhibitors. Nitrate production was depressed when *Hyparrhenia* root macerate was added to soils, but Purchase puts this down to immobilisation of the mineral nitrogen by the presence of root tissue, since there was no build-up of ammonium ions which one might expect if the nitrifying bacteria were suppressed. Purchase considers the scarcity of nitrifying bacteria in *Hyparrhenia* grassland soils to be due to the immobilisation of ammonium ions by root

tissue, hence robbing the microbes of a substrate.

Chase, Corke and Robinson (in *The Ecology of Soil Bacteria*, edit. by T. R. G. Gray and D. Parkinson, 593; Liverpool University Press, 1967) have come to the conclusion that in some soils low pH and phosphate deficiency may limit nitrification. Using perfusion techniques they showed that nitrification could be induced in infertile grassland soils, perfused with ammonium sulphate, by the addition of lime and phosphate. Purchase (*Plant Soil*, **41**, 541; 1974) has also investigated the possibility that the savanna grassland soils could be deficient in nitrifying bacteria because of low phosphate levels, even when ample ammonium ions are available. He added ammonia to soils with varying degrees of phosphate deficiency and found that the nitrification resulting was closely related to the amount of available phosphorus in the soil. He concludes that phosphate deficiency in savanna soils could account for the restricted nitrification, the nitrite oxidisers being particularly sensitive. It is also possible that the grass roots themselves, together with associated rhizosphere microorganisms, are competing with the nitrifiers for phosphate, thus aggravating the deficiency. Robinson (*Plant Soil*, **19**, 173; 1963) has suggested this sort of competition, but for ammonium ions, in New Zealand grasslands.

It is now well known that many higher plant species are able to absorb and utilise nitrogen in the form of ammonium ions. Recent work by Havill, Lee and Stewart (*New Phytol.*, **73**, 1221; 1974) suggests that in some habitats where nitrate production is particularly low, such as acidic, ombrotrophic mires, many of the higher plants have a reduced capacity for nitrate utilisation, since their nitrate reductase activities are very low. Such species must depend upon ammonium ions as their major source of nitrogen. As Rice and Pancholy have pointed out, this mechanism not only bypasses the microbial nitrification stage, which may reduce the risk of nitrate loss from the ecosystem, but also saves the plant the energy required for the activity of nitrate reductase. Whatever the mechanism whereby nitrification is suppressed in some ecosystems, and it is likely that the precise nature of the suppression varies from one ecosystem to another, the resulting nitrate scarcity has evidently placed a selective pressure upon the resident plant species, resulting in their ability to use ammonium ions. This evolutionary response and the suppressed nitrification which has engendered it, may well provide a mechanism for ecosystem nitrogen conservation.

articles

Geology, fauna and palaeoenvironments of the Ngorora Formation, Kenya Rift Valley

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The Ngorora Formation occurs within a long sequence of stratigraphic units that are firmly calibrated by $^{40}\text{K}/^{40}\text{Ar}$ dates. It contains an impressive fauna and has yielded a valuable amount of information on local palaeoenvironments and on the sedimentary processes operative during its deposition. Here, a previously unpublished vertebrate and invertebrate faunal assemblage between 9 and 12 Myr old is recorded, and the stratigraphic framework of this former gap in the fossil mammalian record of Africa south of the Sahara is described.

RESEARCH on the Ngorora Formation¹⁻⁷ has produced a detailed but as yet incomplete picture of the palaeoenvironments and fauna. Key marker horizons have been traced from area to area, detailed measurements have been made of numerous well exposed sections and facies variations have been recognised over a large area. Of particular faunal interest are the collections of Equidae, Hippopotamidae, Orycteropodidae, Hyaenidae, Hyracoidea, Gomphotheriidae and Canidae. Antlered ruminants similar to forms from Fort Ternan have been found, and remains of a small listriodont suid are relatively common.

Palaeogeomorphological setting

The Ngorora Formation was deposited in a basin floored by rocks of the Tiim Phonolites Formation, and was limited to the west by the Elgeyo Escarpment, to the east by a rise towards the area of the present Laikipia Plateau and to the north by the uplands round the Tiati volcanic centre. To the south, the boundary was controlled by the rise of the rift floor, in a similar way as at present (Fig. 1). The basin was tectonically active before, during and after the deposition of the Ngorora sediments. The principal structural trends within the basin are exhibited by escarpments along the Kito Pass, Saimo, Cherial and Kaption faults.

The Saimo Horst was a topographic high during most of Ngorora times and underwent contemporaneous weathering and erosion for at least part of the time. The sediments thin rapidly north-eastwards towards Sidekh, which was also a strong positive element in the topography and is unlikely to have been completely covered by sediment or water during the deposition of the Ngorora Formation.

These two upland areas were separated by about 8 km of low lying country near Bartabwa (C5, Fig. 2) which was mantled by an extensive cover of sediments (Fig. 2). The Ngorora Basin may be divided into three areas on the basis of the types of sediment they contain (Fig. 1). Area I, which lies to the north of the Saimo Horst, comprises two subdivisions. The first is the type area of Kabarsero (area Ia)

lying to the east of the Kito Pass Fault. The second area lies on the western, upthrow side of the fault, where the sequence is thinner (area Ib). Area II comprises the slope of the Tugen Hills west of the Cherial Fault and contains the Kapkiamu Graben and the Kaption Volcanic Complex. Area III lies to the south of the Saimo Horst and contains the upper part of the Ngorora succession lying on a deeply weathered surface of Tiim Phonolite. Correlations between the three areas must remain tentative because of lack of continuity of outcrop between them and the absence of marker horizons common to them all.

Stratigraphy

The thickness of the Ngorora Formation varies greatly in different parts of the basin. At Kabarsero, the type area, the sediments are 400 m thick whereas in area Ib, at Kalimale they are 215 m thick (Fig. 3). The formation thins rapidly north-eastwards from Kalimale (C6, Fig. 2) and pinches out below the summit of Sidekh. In the south, in area III, only the upper parts of the formation are present, and these thin southwards and pinch out at about 0°30'N.

Bishop and Chapman¹ divided the formation into five units which are here ranked as members (Fig. 3). The lowest unit, member A, consists in area I of thick beds of coarse volcanoclastic sediments and clays. Some of the beds originated as lahars, possibly from the Kaption Volcano, lying in area II to the south-west.

Member B consists of alternating, current bedded, gritty tuffaceous beds, clays and silts. An accretionary lapilli tuff in area I may be correlated tentatively with a very thick unit of similar lithology in area II. On the upthrow side of the Kito Pass Fault (area Ib) the clays and silts found in the type area are absent and beds of sandy and gritty tuffs lie directly on top of one another.

In area Ia, member C is composed of finely laminated clays and shales with occasional sand grade beds intercalated. On the upthrow side of the Kito Pass Fault (area Ib) the latter part of member C is cut out by slight erosion and channelling. In area II over 200 m of alternating shales and laminated clays are found in the Kapkiamu Graben. The shales consist almost entirely of chemical precipitates, and the clays were probably produced largely by weathering of the Tiim Phonolites. In area III an unknown thickness of diatomaceous suncracked shales overlies a weathered surface of Tiim Phonolites, but it is not known whether these are the lateral equivalent of member C.

In areas I and II, member D is a succession of clays rhythmically alternating with cross-bedded silty and gritty tuffaceous beds. Earth movements during the deposition of member D are indicated by slumps, faults and fissures which do not affect the overlying units. At Cheprimok (C5, Fig. 2) major faulting resulted in the erosion of nearly 130 m of

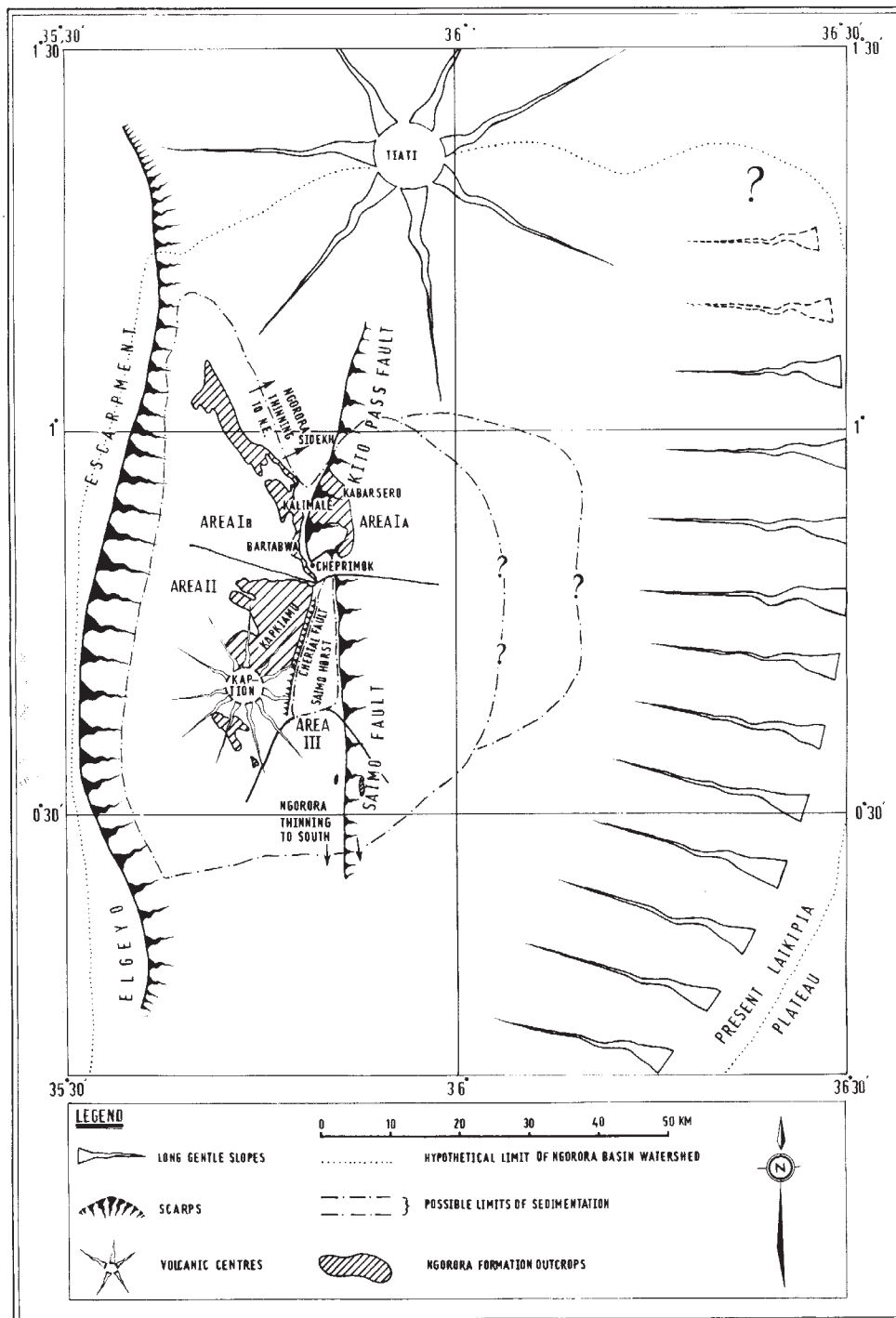


Fig. 1 Regional setting and outcrops of the Ngorora Formation.

sediment so that member E rests unconformably on member A and oversteps on to Tiim Phonolite. This faulting episode was widespread and has been recognised in area II, 20 km away. In area III a series of red marly earths and silts may be the lateral equivalent of member D.

Member E sediments were deposited after another major faulting episode which lowered the base level to such an extent that lacustrine conditions were established again. A series of diatomaceous and fish-bearing shales is widespread over the whole of area I and parts of area II, but erosion has removed much of the record. The erosion was consequent on movement on the Kito Pass and Saimo Faults which uplifted the crest of the Tugen Hills tilt block until it was exposed to subaerial processes. Area Ia, on the down-

throw side of the faults, underwent further sedimentation but the changed environment resulted in the deposition of coarse material including conglomerates, rather than the deposition of shales like those found in the lower part of the sequence. In area III, a series of conglomerates and bedded tuffs may be equivalent to member E. In every area an erosional unconformity separates member E from the overlying Ewalel Phonolite Formation.

Figure 3 illustrates stratigraphic sections from areas Ia and Ib respectively, to show variation in thicknesses of the different members. Several important horizons can be traced across the Kito Pass Fault and reveal thickness changes indicating contemporary fault movement throughout the deposition of the sequence. The units on the upthrow side

of the fault are usually condensed sequences, whereas those on the downthrow side often contain extra units of clays and silts.

Sediments are derived from several sources. The Kaption Volcano in area II probably supplied many of the lahars and much of the tuffaceous material found in area I. Some sediment was derived from the Laikipia area and also from the Tiati volcanic centre. The laminated clays of the Kapkiamu Graben may have been derived from phonolite weathering products on Saimo Horst.

The Saimo Horst and Sidekh tilt block acted as barriers to sediment derived from the east, although the Bartabwa gap may have functioned as a funnel for sediment crossing the Kito Pass Fault. Channel deposits in area Ia to the east of the line of the Kito Pass Fault are more fossiliferous (786 specimens from 19 localities) than those in area Ib to the west (59 specimens from 11 localities).

Fauna and faunal correlation

An extensive collection of fossils was made during the 1972-73 field season. It included 1,400 fragments of mammals, 500 fragments of fish, 3,000 mollusca and a variety of less common fauna and flora, from 132 fossiliferous localities. Many of the specimens have not yet been studied in detail but preliminary identifications are presented in the faunal list (Table 1). Figure 4 is a pictorial representation of the stratigraphic ranges of the various faunal and floral elements.

A tentative correlation with the uppermost Vindobonian has been attempted³ on the basis of the fauna and particularly on the apparent absence of *Hipparion*. The discovery of *Hipparion* in members D and E resolves much of the speculation over the apparent absence of Equidae from sub-Saharan Africa at this early date. Hooijer⁸ has concluded that the Ngorora Equidae are the same species as the earliest equids of northern Africa, and Eurasia.

The Ngorora Formation is younger than the Fort Ternan Formation from the evidence of radiometric dates and mammalian faunas. The Bovidae seem slightly more advanced than their Fort Ternan counterparts (A. Gentry, personal communication). A Vindobonian age is clearly too old and the Ngorora Formation is probably equivalent to the Vallesian and Lower Turolian of Europe and possibly the Chinji/Nagri Stages of the Siwalik Series.

When additional dates have been established for tuff horizons in the sediments it should be possible to estimate more precisely the age of these earliest sub-Saharan Equidae. At present, whole rock ⁴⁰K/⁴⁰Ar age determinations suggest ages of ~12 Myr for the underlying Tiim Phonolite and ~8.5 Myr for the overlying Uwalé Phonolite^{2,6}. Sanidine feldspars from member B have yielded ages of ~11.94 and ~12.31 Myr and ages of ~9.82 and ~9.68 Myr from member D (J. A. Miller, personal communication).

The oldest known remains of Hippopotamidae occur in the Ngorora Formation². The number of specimens has been significantly increased by finds during the latest field season. Other interesting elements of the fauna include a hominoid, a cercopithecoid, a new genus of large hyracoid, a new species of gomphotheri⁷, a canid, a mellivorine mustelid, a new species of *Orycteropus*, the ruminants *Protragocerus*, *Pseudotragus*, *Palaeotragus*, *Gazella*, *Samootherium*, and an antlered form, several rodents and a small listriodontine suid. A hyaenid has also been recorded. A large variety of molluscs has been collected, and impressions of several types of insect have been found, including termites, mosquitos, moths, and flies. Of striking interest is the great difference between the rhinocerotid faunas of Ngorora⁴ and those of Fort Ternan. This is strange in view of the similarity between the ruminants found at both localities.

Several earlier determinations of taxa are incorrect. Chalicotheriidae¹ is now recognised as Hyracoidea.

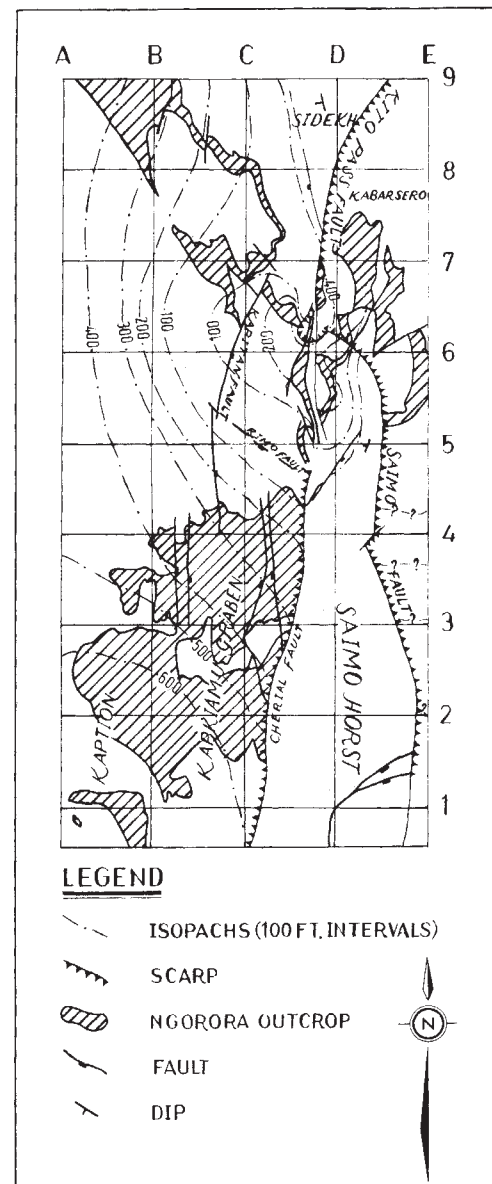


Fig. 2 Thickness variation and major structural controls, Ngorora Formation.

*Chilotherium*² should be *Chilotheridium*⁴. Tapir³ should be Listriodontinae, and *Deinotherium hobleii*² should be *Deinotherium bozasi*.

Various plant fossils have been collected, including wood, leaves and seed pods. Among the Protista the diatom *Melosira* sp. is recorded, and calcareous algae are common. Among the Trachaeophytes, both Monocotyledons (palms and grasses), and Dicotyledons (lianas, *Celtis* sp.) and several others are represented. A bullrush-like plant was found in member B. No pollen has been found.

The Ngorora fauna is not restricted to a single small locality as is that at Fort Ternan, but occurs over a large area. It spans a period possibly as long as 3 Myr. Its value is enhanced by the detailed palaeogeomorphological and palaeoenvironmental evidence that can be deduced for the formation (Fig. 4 and Table 2).

Palaeoenvironment

Reconstructions of palaeoenvironments often rely heavily on fossils, and thus engender circular arguments about the habitats occupied by different elements in the fauna. Apart from details of the topography of the basin floor already

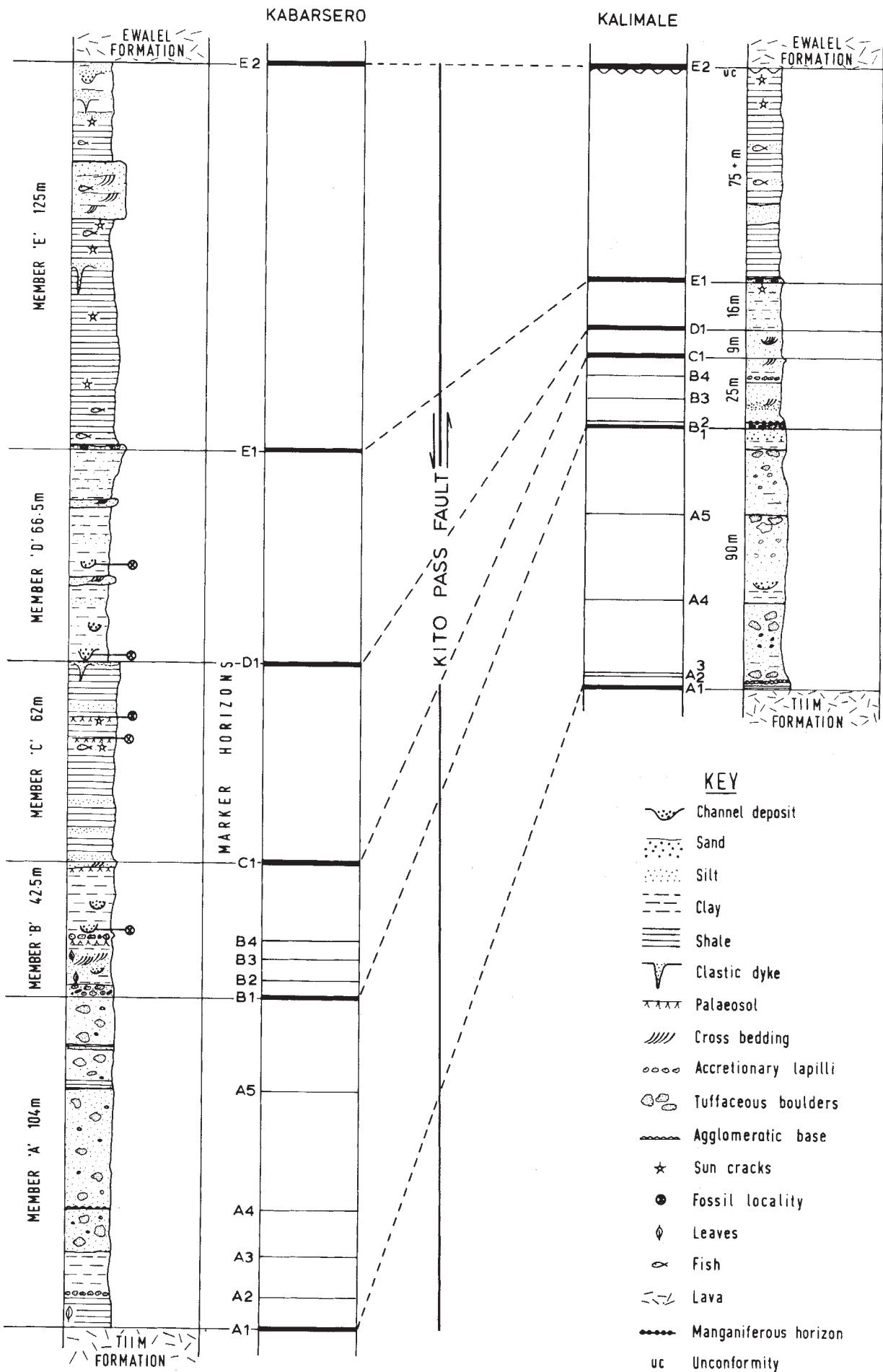


Table 1 Faunal list from the Ngorora Formation

Mollusca		Primates	
Gastropoda		Cercopithecoidea	indet.
Prosobranchia	<i>Lanistes</i> sp.*	Hominioidea	indet.
	<i>Bellamya</i> sp.*	Rodentia	Dendromurinae gen. nov.*
	<i>Melanoides tuberculata</i> *		Cricetidae gen. nov. small*
	small sp.*		gen. nov. medium*
Pulmonata	sp. indet.*		Phyomyidae gen. nov.*
Bivalvia	<i>Pleiodon</i> sp.*		?Peditidae*
	<i>Mutela</i> ? sp.*		?Sciuridae*
Annelida	indet.*	Carnivora	
Arthropoda		Canidae	gen. nov.*
Insecta		Mustelidae	<i>Mellivora</i> sp. nov.*
Isoptera	indet.*	Hyaenidae	<i>Percrocuta tobieni</i> Crusafont & Aguirre
Lepidoptera	indet.*		
Diptera	indet.*	Tubulidentata	
Coleoptera	indet.*	Orycteropodidae	<i>Orycteropus</i> sp. nov.*
Crustacea		Proboscidea	
Ostracoda	<i>Heterocypris</i> sp?*	Gomphotheriidae	<i>Gomphotherium ngorora</i> Maglio
	<i>Candona</i> sp.*	Deinotheriidae	<i>Deinotherium bozasi</i> Arambourg
	<i>Metacypris</i> sp.*	Hyracoidea	
	<i>Limnocythere</i> sp.*	Procaviidae	<i>Paraplioxyrax</i> sp. nov.
	<i>Potamon</i> sp.*	Perissodactyla	
Decapoda		Equidae	<i>Hipparion primigenium</i> von Meyer
Pisces		Rhinocerotidae	<i>Chilotheridium pattersoni</i> Hooijer
Cypriniformes			<i>Aceratherium</i> or <i>Dicerorhinus</i>
Cyprinidae	indet.*		<i>Brachypotherium</i> sp.
Clariidae	c.f. <i>Clarias</i> sp.		
Perciformes		Artiodactyla	
Cichlidae	<i>Tilapia</i> sp. nov.	Suiformes	
Reptilia		Suidae	gen. et sp. nov.*
Chelonina		Listriodontinae	c.f. <i>Hippopotamus</i> indet.
Testudinidae	<i>Testudo</i> sp.*	Hippopotamidae	gen. nov. indet.
Trionychidae	<i>Trionyx</i> sp.		
Pelomedusidae	<i>Pelusios</i> c.f. <i>sinuatus</i> Smith	Ruminantia	
Crocodylia		Tragulidae	2 spp.
Crocodylidae	<i>Crocodylus</i> sp.	Giraffidae	<i>Palaeotragus</i> sp.
Squamata			? <i>Samotherium</i> sp.*
Varanidae	indet.*		indet.
Ophidia	indet.*	Lagomerycidae	<i>Protragocerus</i> sp.
Aves		Bovidae	Boselaphini/Tragelaphini sp.
Struthioniformes			or spp.
Struthionidae	indet.*		?Cephalophini sp.
Ciconiiformes			? <i>Pseudotragus postpotwaricus</i>
Ciconiidae	<i>Leptoptilos</i> sp.		Gentry
Mammalia			Neotragini sp.*
Insectivora			<i>Gazella</i> sp.
Macroscelididae	indet.*		

*Previously unrecorded from the Ngorora Formation.

We thank the following for studying specific elements of the fauna: Dr A. Gentry (Bovidae); Dr R. Hamilton (Giraffidae and Tragulidae); Mrs S. C. Savage (Hippopotamidae); Dr Bate (Ostracoda); Dr A. Gautier (Mollusca); Dr J. J. Jaeger (Rodentia); Dr V. J. Maglio (Gomphotheriidae); Dr D. A. Hooijer (Perissodactyla); Dr H. Osmaston (fossil leaves); and Mr D. Brett (fossil wood).

discussed here, the Ngorora sediments yield evidence of a variety of conditions prevailing both during and after deposition. Palaeoenvironmental indicators in the sediments include polygonal desiccation cracks, calcretes and other palaeosols, *in situ* plant remains, channelling and cross-bedding, bird footprints, evaporite deposits, rhythmic units, algal and oolitic limestones, subaerial tuffs, diatomites, chert nodules and other structures. Table 2 summarises the evidence for the five members in three different areas.

Member A predominantly comprises coarse, reworked pyroclastic material, with some primary elements (for instance around the Kaption volcanic centre). The coarse beds contain very few fossils, but the thin shaly horizon at the bottom of the type section contains rhizomes in position of growth. At Chepchomus (C6, Fig. 2), a few proboscidean and rhinocerotid remains were found, resting on the Tiim Phonolite. At the top of member A some grit-filled channels occur, but these were poorly fossiliferous and yielded only a few reptilian fossils together with comminuted fish bones.

During the deposition of member B, Kaption Volcano

was still intermittently active and was quite possibly a source of the accretionary lapilli tuff, one of the most reliable marker horizons in the Ngorora sequence. In area 1b, there were several episodes of emergence of the land surface giving rise to palaeosols and weathering profiles. In particular, there is a widespread pyrolusite nodule horizon in the Bartabwa area (C5, Fig. 2) which seems to have been formed by subaerial weathering of the underlying tuffaceous bed. It was subjected to contemporaneous channelling and winnowing, as evidenced by thinning of the unit and local concentration of the nodules into a conglomerate. The accretionary lapilli tuff was deposited after the formation of the pyrolusite horizon, and soon acquired an active and flourishing plant growth, as shown by numerous rootlet casts. In places, as at locality 2/1 (Kabarsero), it was channelled and potholed by running water. A mile south of Bartabwa, casts of rhizomes occur in the accretionary lapilli tuff and at Kalimale (C6, Fig. 2) plant growth and weathering were vigorous enough to destroy all the lapilli in a bed 45 cm thick, giving rise to an extensive calcrete (caliche) horizon. Fortunately, patches of the original material have survived, thus allowing stratigraphic correlation.

The varied fauna recorded from member B is of limited

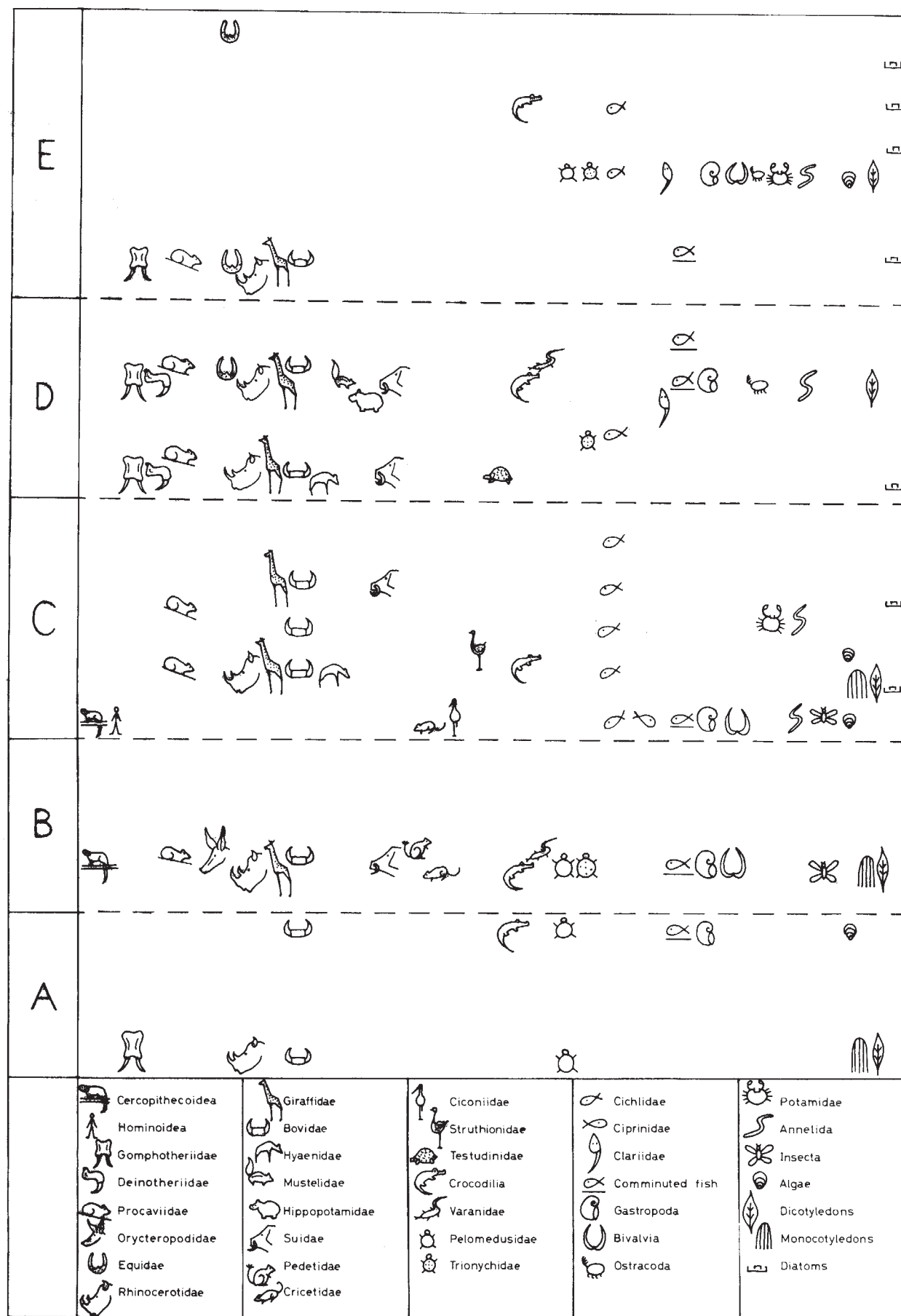


Fig. 4 Distribution of major faunal elements, Ngorora Formation.

use in deducing the nature of the local palaeoenvironment, as nowhere is there a 'life assemblage'. It could have been derived from many miles away, and from several different environments. The ruminants, however, indicate an open or lightly wooded grassland habitat⁵.

Kaption Volcano had ceased activity by the beginning of deposition of member C which was heralded by large scale faulting along both the Kito Pass-Saimo Fault system, and the Cherial-Kaption system. Three lakes were formed, one at Kabarsero (area Ia), one in the Kapkiamu Graben (area II), and the third to the west of the Kaption Complex. The first was a fresh water lake, as shown by the diatoms and crabs, and could have been well oxygenated. Several regressions occurred and the sediments became suncracked and fissured, with the development of palaeosols. Some of the palaeosol horizons have yielded fossils, including articulated bones and, in one case, a complete skeleton. These occurrences are more likely to represent life assemblages than are those of members B and D. Taphonomic studies are being undertaken to determine the main differences between the two types of deposit. Potamid crabs indicate that the water of the Kabarsero lake was fresh. The Kapkiamu lake on the other hand was most probably an alkaline body of water; the shales were probably chemical precipitates (Judith Van Couvering, personal communication). A new form of stunted *Tilapia* is found in abundance in the shales, and is similar to *Tilapia grahami* found at the present day in the soda lake Magadi (Judith Van Couvering, personal communication). Beds of algal and oolitic limestone that give off a petroliferous odour when freshly broken also occur. They usually overlie suncracked horizons.

The shales and laminated clays were laid down in rhythmic fashion and possibly indicate seasonal fluctuations of climate. Both units are finely laminated, and record periodic increments of sediment. Fossil wood from member C contains well developed growth rings suggestive of fluctuating climatic conditions.

The juxtaposition of fresh and saline lakes comes as no surprise as there are two such systems in existence in the Kenya Rift Valley at the present time. Lakes Baringo and Hannington lie about 20 km apart and lakes Naivasha and Elementaita lie about 25 km apart. The palaeolakes of Kabarsero and Kapkiamu were about 8 km apart. The area between them was one of low ground upon which a vigorous plant cover and palaeosols were developed. Contemporaneous erosion was occurring and in places channels are seen, but the relief was, in general, low. Some manganiferous weathering horizons were developed during this time, and it is above one of these, at locality 2/9 (C5, Fig. 2), that the hominoid molar was found. The third lake, west of Kaption, contained freshwater.

Member D began with rejuvenation which resulted in channelling, and the deposition of grits and conglomerates and coarse tuffaceous beds. There is a marked decrease in grain size to the west. Clay horizons are intercalated between the coarse units in a manner similar to those of member B. Unfortunately, landslide and scree obscure much of member D in area Ib, but in area II there are several good exposures which contain suncracked horizons, palaeosols and other evidence of emergence or shallow water. Crabs, ostracods and fish are found at Kalimale (C6, Fig. 2) in a marginal lacustrine deposit, indicating freshwater conditions nearby.

Scree and landslide obscure much of member E in area Ib, and in area II the unit has been eroded away almost completely. The lower part of the member is composed of well laminated, diatomaceous shales with numerous suncracked layers. The development of clastic dykes and 'sills' is in places spectacular. At Cheprimok (C5, Fig. 2) sedimentary rhythms are well developed with up to nine units comprised of a coarse volcanoclastic and fish-bearing lower part, followed by a shaly, suncracked portion. Zeolite formation is common and palaeosols are developed on some units. One

of these palaeosols contained the remains of a new gomphothere⁷. The lacustrine episode of member E was terminated by renewed movement along the faults which resulted in the lowering of area Ia and the uplifting of areas Ib and II which began to undergo erosion. In area Ia sedimentation was continuous, but the energy of the environment had changed so that grits and conglomerates were deposited above the shales.

Table 2 Summary of palaeoenvironmental reconstructions, Ngorora Formation

Member	Area Ia	Area Ib	Area II
	Rejuvenation, giving rise to conglomerates and grits.	Uplifted by faulting with consequent erosion.	
E	Lacustrine, fresh water with numerous lake oscillations producing suncracks and clastic dykes.	Lacustrine, fresh water, well developed rhythmic units caused by lake oscillations. Some calcretes formed.	Record removed by erosion.
D	Fluviatile with several prolonged emergences marked by palaeosols. Ruminants suggest open or lightly wooded areas.	Marginal lacustrine and fluviatile; fresh water. Fluctuating lake levels with frequent desiccation.	Lacustrine environment with minor fluviatile episodes. Some palaeosols and desiccation cracks developed.
C	Freshwater lacustrine environment. Oscillating lake levels with prolonged emergences marked by palaeosols. Growth rings in wood suggest fluctuating climate.	Predominantly emergent with weathering and erosion. Minor paludal facies. Low relief.	Alkaline lacustrine conditions with very low energy. Many lake level fluctuations. Rhythmic units developed, with possible climatic control.
B	Fluviatile with some prolonged emergences and development of palaeosols. Possibly two primary tuffs. Vigorous plant growth.	Predominantly emergent with well developed palaeosols. Otherwise fluviatile. At least one primary tuff. Vigorous plant growth.	Little evidence, owing to proximity to volcanic centre. Thick primary tuffs.
A	Lacustrine at first followed by several lahars, primary agglomerates and tuffaceous sediments. Minor fluviatile facies.	Lahars, tuffaceous sediments; minor fluviatile facies.	Primary volcanic activity, near eruptive centre; tuffs, agglomerates and lavas.

The sedimentary sequence is overlain by lavas of the Ewale Phonolite Formation which flowed north and covered much of the Ngorora Basin. Continued faulting along the Kito Pass and Saimo Faults, and later erosion led to re-exposure of the sediments. The area was then covered by the Kabarnet Trachyte Formation, K-Ar dated at about 7 Myr (ref. 2). Further faulting, in particular during the Pleistocene, resulted in the uplift of the Tugen Hills to their present height, and re-exposed the Ngorora Formation.

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Structure of tobacco mosaic virus at 6.7 Å resolution

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The electron density distribution of tobacco mosaic virus has been determined to 6.7 Å resolution by analysis of the X-ray diffraction pattern given by oriented gels of the virus. This has been achieved by separation of overlapping Bessel function terms by a technique analogous to crystallographic isomorphous replacement. The course of the polypeptide chain of the coat protein may be traced for a large part of its length.

TOBACCO mosaic virus (TMV) consists of long, rod-like particles which can be made to form large regions of oriented gel in a capillary tube. These gels give a detailed diffraction pattern (Fig. 1), as was shown by Bernal and Fankuchen¹. This demonstrates that TMV is periodic, repeating every 69 Å with a pseudo-repeat every 23 Å. The theoretical development by Cochran, Crick and Vand² of the diffraction pattern of a helix allowed Watson³ to recognise that the diffraction pattern given by TMV gels was a typical helix diffraction pattern and moreover that the repeating unit of TMV must contain $3n + 1$ subunits in three turns. It was later shown by Franklin and Holmes⁴ that there are 49 subunits in three turns. Franklin⁵ and Caspar⁶ independently used the method of isomorphous replacement applied to the zero layer line of TMV to calculate a radial density distribution of the virus, and Franklin⁵ showed that the RNA is at a radius of 40 Å. Klug (unpublished) later confirmed that this is a single strand winding along the basic helix, by studies of the other layer lines.

Because the TMV particles are randomly oriented about their long axes, one obtains in a diffraction experiment the cylindrical average of the square of the Fourier transform of a single virus particle. Interparticle interference may be neglected, so the intensity is continuous. Franklin and Klug (unpublished) demonstrated that it should be possible to extend the method of isomorphous replacement as classically used in protein structure analysis to deal with the situation encountered in TMV. A number of theoretical and technical problems impeded the progress of this research and our paper represents the fruition of this attempt to determine the structure of TMV from oriented gels using the method of isomorphous replacement.

Major sources of difficulty

The amount of data available is limited and errors tend to be systematic, so that extremely accurate measurement and correction of data is required.

The main problem encountered concerns the location of heavy atoms. Because of the very high symmetry of TMV (49-fold) and the low resolution data available, Patterson methods were early shown to be impractical. Automatic search methods based on *R*-factor minimisation using single heavy atom derivatives gave ambiguous results. The use of double heavy atom derivatives together with the single heavy atom

derivatives can partially remove these ambiguities⁷ but a full scale *R*-factor search using five heavy atom derivatives and two double derivatives, and more accurate data was required in this work to yield unambiguous heavy atom positions. These methods are described briefly below.

The problem of cylindrical averaging is solved in two stages. At low resolution (10–15 Å) the diffraction intensities are unaltered by the averaging, because of the high symmetry of the TMV particle. Therefore it is possible to reconstruct the electron density of virus by establishing the phases of the observed intensities by the normal method of isomorphous replacement. At higher resolution new methods have been required, based on an extension of the isomorphous replacement method.

Data collection

Data were collected with a double-focused monochromatised beam (see Fig. 1) and a computer-controlled densitometer. The major corrections were background subtraction and a correction

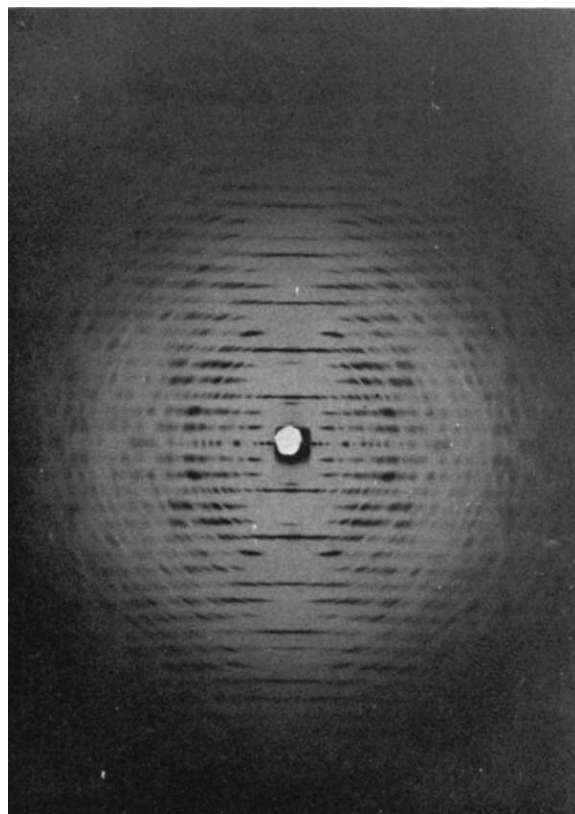


Fig. 1 A fibre diffraction pattern from an oriented gel of TMV. The exposure time was 87 h in an evacuated Guinier focusing camera (specimen film distance 11.2 cm) using a point focused beam from two bent quartz focusing monochromators and an Elliott fine focus rotating anode tube.

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Table 1 Symbols used in this paper

R , Reciprocal space radius
l , Layer line index
c , Axial repeat (69 Å in TMV)
r , Real space radius
ϕ , Real space azimuthal angle (cylindrical coordinates)
z , Real space vertical coordinate
f , Atomic scattering factor
F , Structure factor
J_n , Bessel function, order n

for disorientation. Automatic background subtraction had not been successful, because arcs from strong spots extend into the region between layer lines where background is measured. But an interactive computer display system using manual intervention where necessary to override automatic procedures has proved adequate. These and other developments are described by Barrington Leigh⁸ and Mandelkow⁹. The correction for disorientation has been described by Holmes and Barrington Leigh¹⁰ and Stubbs¹¹.

Location of heavy atoms

A summary of previous attempts to locate the heavy atoms is given in ref. 7. The use of single site substitutions was of paramount importance in the initial location. The heavy atoms were located in three stages:

Determination of r and f . Radius and occupancy are the only parameters which affect the intensity on the inner centric part of the zero layer line. They may be determined by comparing the difference between the native and derivative structure factors (G) with the expected structure factor of a heavy atom ($fJ_0(2\pi Rr)$). The parameters are refined by a least squares procedure. An exact description of the process is given by Mandelkow and Holmes¹⁵.

Determination of ϕ and z . It can be seen from equation (2) that, for a given Bessel function term, the phase Φ of the contribution of one atom to G is a constant

$$\Phi = -n\phi_j + 2\pi lz_j/c$$

where r_j , ϕ_j , z_j are the cylindrical polar coordinates of the heavy atom. Φ may be found by a search procedure⁸, in which the least-squares R -factor for each layer line

$$R = \sum (|F_H|_{\text{obs}} - |F_H|_{\text{calc}})^2$$

is minimised with respect to Φ . The values of Φ found from different layer lines can be combined to find ϕ_j and z_j . The procedure is limited to the regions of the diffraction pattern where G s do not overlap.

Refinement. Refinement analogous to conventional crystallographic heavy-atom refinement¹⁶ was carried out on the heavy atom r , ϕ and z coordinates by an extension of the process outlined above. There were insufficient data to refine occupancy or temperature factor.

Cylindrical averaging

The structure factor of a helix of repeating subunits was first evaluated by Cochran, Crick and Vand². From their results, Waser¹² and Franklin and Klug¹³ calculated the cylindrically averaged intensity. In the notation of Klug, Crick and Wyckoff¹⁴ this is

$$I = \sum_n G_{nl} G_{nl}^* \quad (1)$$

where

$$G_{nl} = \sum_j f_j J_n(2\pi Rr_j) \exp(-in\phi_j + 2\pi lz_j/c). \quad (2)$$

The summation in equation (2) is over all the atoms in the asymmetric unit. The notation is given in Table 1.

The summation over n in equation (1) is confined to those values of n which obey the helix selection rule

$$l = tn + um$$

where the helix has u subunits in t turns (49 and 3 for TMV); m is any integer. Since a Bessel function has a very low value until the argument is nearly equal to the order only a small number of different orders (and thus G terms) can contribute to the intensity at low resolution. For TMV to a resolution of about 12 Å, there is only one term, and two terms suffice for a resolution of 6–7 Å.

To perform a Fourier inversion and thus obtain an electron density map, the real and imaginary parts of each G must be known. The problem may be formulated in a way analogous to the classic crystallographic phase problem but with three unknown parameters rather than one. These may be considered as the phase of each of two terms originating from different

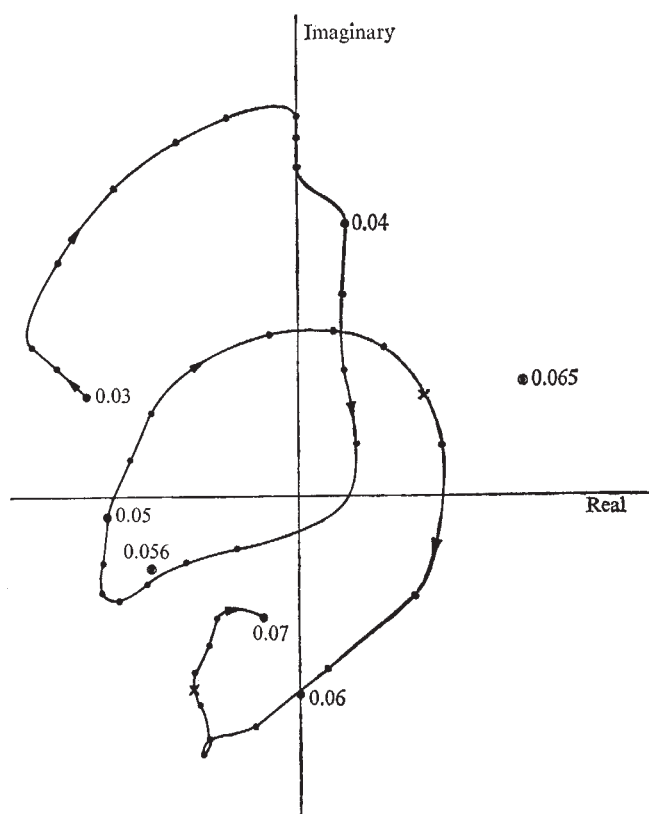


Fig. 2 Continuity of the transform of TMV, for layer line 1, $R = 0.030$ to 0.070 . Each dot represents the value of the most probable G on the Argand diagram. The line connects dots adjacent in R . Circled dots do not fit the general continuity. The crosses represent minor peaks in the probability distribution of such discontinuous points, which do fit the continuity. The points represented by crosses are therefore accepted as the value of G to be used, rather than the circled dots. Argand diagrams such as this have previously been used by A. Klug (personal communication).

order Bessel functions (see equations (1) and (2)) and the ratio of their amplitudes, which for convenience is often expressed as the tangent of a third angle. The problem is equivalent to searching over the surface of a four-dimensional hypersphere using four or more heavy atom derivatives to determine the correct hyperspherical (three component) phase angle. In practice, however, it is more convenient to determine the G parts directly. They can be found by the method of isomorphous replacement. If a heavy atom is bound to the structure, and its position is known (see below), its contribution to G can be

Table 2 Heavy atom derivatives

Ligand	Strain	Residue	<i>f</i>	<i>r</i> (Å)	φ(°)	<i>z</i> (Å)
DMA	Ni-2068	139	40	72	0	0
MMN	<i>Vulgare</i>	27	32	56.7	16.0	11.7
OsO ₄	<i>Vulgare</i>	1?	35	92.5	5.7	9.4
SHIMS-MMN	<i>Vulgare</i>	68(+27)	25	71.1	5.3	-1.3
Pb ²⁺	<i>Vulgare</i>	115/116	24	25.4	7.1	20.6
Double derivatives (excluding SHIMS)						
DMA/Os	Ni-2068	139/1?				
MMN	Ni-2068	139/27				

SHIMS, sulphhydryl imidoester²⁷; DMA, dimercury acetic acid; MMN, methylmercury nitrate⁴. Units of *f* are arbitrary, and correspond to approximately one-third of an electron.

calculated. If this is $a+ib$, and $G = A+iB$, we have from equation (1)

$$I_H = \sum (A+a)^2 + (B+b)^2 \quad (3)$$

To solve unambiguously for all *A*s and *B*s, as many heavy atom derivatives are required as there terms in the summation in equation (3). The solution may then be found numerically, by a procedure quite analogous to conventional phase determination. This requires a great deal of computing, however, so an analytical procedure has been developed (Diamond and Stubbs, unpublished, based on the usual assumption that there are no errors in native intensities. With this assumption a series of equations of the type (3) may be reduced to linear equations, errors in which are minimised subject to the non-linear constraint implied by equation (1). The results will usually take the form of the peaks of a probability distribution for the pairs (*A*, *B*). It would be quite possible to accept the most probable set of *A*s and *B*s, treating each measured point on the fibre diagram separately. But to do so would be to neglect an important constraint on *G*.

The continuity constraint

As mentioned above (equation (2)), *G* is a continuous function of *R*, and the restriction $r_{max} < 90 \text{ Å}$ (the maximum radius of the particle) puts a limit on the rate of fluctuation in *G*. If, for example, a series of points along a layer line have smoothly varying values of *A* and *B*, except for one point, a smaller peak in the probability distribution of the discontinuous point often corresponds to the value of the smooth curve. This is illustrated in Fig. 2 for the single Bessel function case. The more compli-

cated situations which arise when two Bessel functions are present can be dealt with either in the same way (by inspection), or by a semi-quantitative procedure suitable for a computer (Stubbs, unpublished). Thus, real and imaginary parts for each *G* can be determined which provide an optimum balance between probabilities based on isomorphous replacement data and the constraint of continuity.

The derivatives

Four single-site derivatives and three double derivatives, with a total of five independent sites were used. The double derivatives were originally used to assist in the location of heavy atom sites. In this work, their main function was to reduce errors by providing partially independent extra data, but in the last few rounds of refinement, the methylmercury nitrate site at residue 139 (see Table 2) was freed from the dimercury acetate site. The movement was in fact very small.

Table 2 summarises the information on the derivatives. Because of the importance of single sites, sulphhydryl-mercury reactions were used whenever possible. The Ni-2068 mutation¹⁷ was used because it contains only one sequence change, Tyr 139 → Cys. The large dimercury acetic acid (DMA) group can react with this residue, but not with Cys 27, which only reacts with small mercurials.

The osmium site is tentatively assigned to the N-terminus, because it is the same as the N-terminus of the strain U2 of TMV¹⁸. This strain differs from *vulgare* in 35 amino acids of the coat protein, but gives a very similar diffraction pattern. The assignment is made plausible by the fact that a difference Fourier between TMV-*vulgare* and U2 shows no significant differences at radii greater than 70 Å. The chemical location of the lead site is uncertain. Butler and Durham¹⁸ concluded that the reaction, which is believed to be with carboxyl groups (see ref. 19), could only be with Asp 115 and Asp 116.

The electron density map

Apart from a short section of layer line 3 where there are three *G* terms (and this section has relatively low intensity so that it could be omitted), only one or two *G* terms contribute to the data to a resolution of 6.7 Å and we were able to evaluate the *A* and *B* parts of each *G* term within this region. This was done

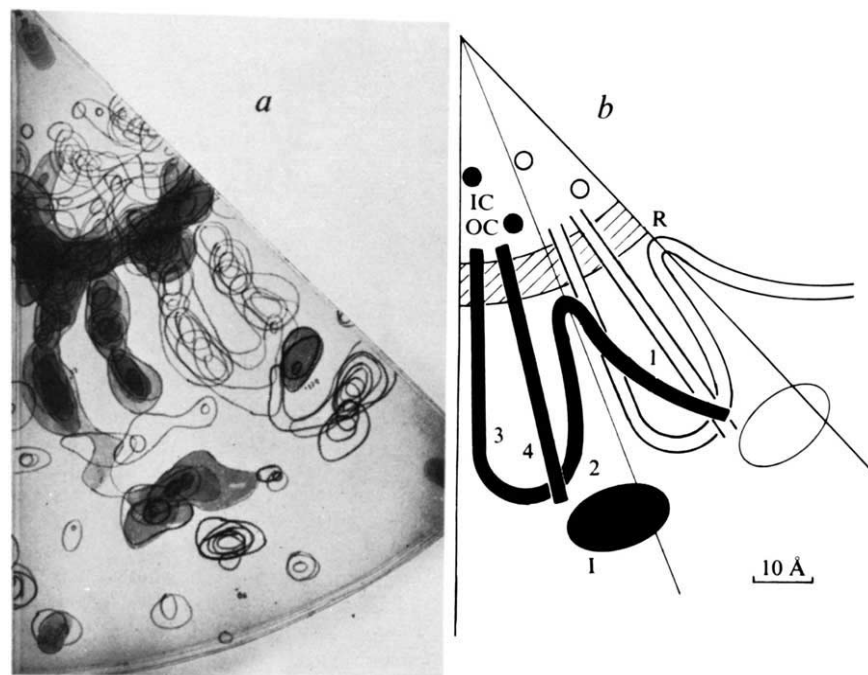


Fig. 3 *a*, Five sections of the electron density map, at 2 Å intervals in *z*. These sections contain two molecules adjacent in ϕ . One molecule and the RNA strand are shaded. Clearly visible are the RNA, parts of ribbons 2 and 3 (see Fig. 4*b*) and the C-terminus region. *b*, Schematic diagram of the main features in the electron density map. The diagram represents two subunits, one being shown by open symbols for convenience. IC, Inner column; OC, outer column; I, island; R, RNA; 1, 2, 3, 4, ribbons.

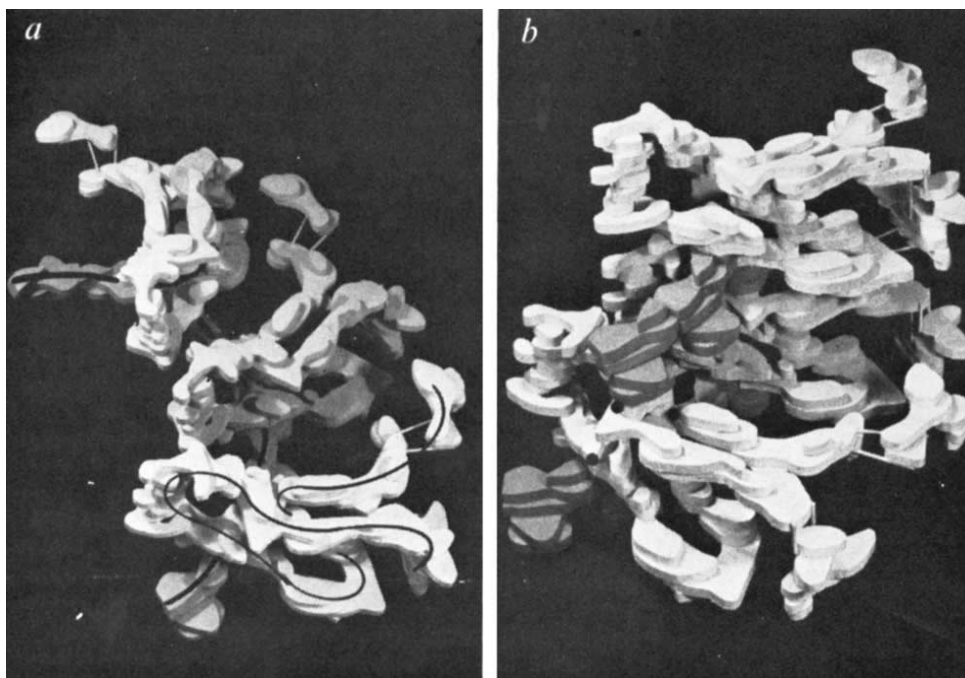


Fig. 4 Balsa wood models of the electron density. *a*, A half circle of RNA is shown (heavy line) with three protein molecules arranged along the TMV helix. Every other subunit has been omitted for clarity. The main chain of the protein is shown (light line). *b*, Three subunits of TMV protein, showing the interaction between subunits in successive turns of the helix. Note the three points of RNA-protein interaction, which are marked (●).

by trapezoidal integration at intervals of $0.001 \text{ \AA}^{-1}$ along each layer line. Compared with the mean wavelength of the Gs $0.001 \text{ \AA}^{-1}$ is a fine interval. A Fourier inversion was computed. Five sections of the resultant electron density map are shown in Fig. 3*a*. Figure 3*b* is a summary of the main concentrations of electron density. The very strong helical ribbon at about $40 \text{ \AA}$ radius may be identified as RNA. The rest of the density, which must be coat protein, can be roughly divided into four approximately radial ribbons between 40 and $70 \text{ \AA}$ radius, two columns of density at 22 and $32 \text{ \AA}$ and an island of density between 75 and $85 \text{ \AA}$. We can use the chemical information available about the protein to trace some of the chain.

Three of the ribbons form one continuous chain of density touching the RNA at two points. Residues 27 and 68 are labelled by heavy atoms, and appear to be in this chain. Since we believe the N-terminus to be at a radius of about $90 \text{ \AA}$ (see Table 2), we continue the chain back from residue 27 into the island, crossing at the only point which avoids very low densities.

Residue 139 is labelled, and is close to the inner edge of the island, so it seems that the C-terminus is also at high radius. This is confirmed by its availability to carboxypeptidase²⁰. Therefore, the fourth ribbon must take the sequence from the RNA interface back to the island, and all the density inside the RNA helix must connect the third and fourth ribbons. This would agree with Butler and Durham's¹⁸ location of the lead on residues 115 and 116.

The inner density is difficult to follow, but a tentative interpretation can be made. It seems that the chain probably runs up the inner column and down the outer, before crossing the RNA to join the fourth ribbon.

An interesting feature of this interpretation is that the island contains the C-terminus of one subunit and the N-terminus of the adjacent subunit. We would emphasise that this feature of the interpretation is somewhat preliminary.

Figure 4 consists of two photographs of balsa wood models of three subunits of TMV protein and strands of RNA. It is clear that the structure is essentially four fairly straight chains, with the RNA in a hollow on top of the molecule.

Conclusions from the map

Even though the map is only at $6.7 \text{ \AA}$ resolution, the following conclusions may be drawn:

First, the map suggests there is little β sheet since this would show up as non-resolved regions of high density at this resolution. Apart from very short sequences, the only place where a

β sheet could be accommodated would be the island, which contains at most 20% of the protein.

Second, there are several regions of high protein density which would be consistent with α -helix, including two regions of high, rod-like density which are very good candidates for helices. These are probably in the vicinity of residues 55 and 95. α -helix was predicted at the first of these residues by Schiffer and Edmundson²¹ and by Leberman²², and Leberman also predicted the second. In fact, all of Leberman's five α -helical regions and five of Schiffer and Edmundson's six regions could be in or near our regions of high density.

Another interesting feature is the points of contact with the RNA. There are three, all in regions which are conserved in many strains and viable mutants of TMV. Furthermore, all three contain invariant arginines (residues 41, 90 and 92, 113) which have often been suggested as possible residues for interacting with phosphate groups.

Finally, the island of density is interesting. It seems to be joined to the rest of the protein by a region of low density which may be a hinge allowing some freedom of movement in the z -direction. Caspar and Holmes²³ concluded from studies of the *Dahlemense* strain that the outermost part of the subunit in *Dahlemense* TMV is periodically perturbed. It is possible that the structure involved in the *Dahlemense* perturbation is the outer island and its associated hinge.

This work is continuing, although the maximum resolution obtainable from this approach is probably only $5 \text{ \AA}$. Klug and his coworkers in Cambridge are working on the crystal structure of the two-layer 17-fold disks of the coat protein²⁴⁻²⁶ and it should be possible to take this work to atomic resolution. Eventually we hope to combine the two maps to obtain an atomic model of the entire virus.

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Control of somite number during morphogenesis of a vertebrate, *Xenopus laevis*

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During Xenopus embryogenesis and early growth, somite number stays close to a species-typical value for each morphological stage. This remains true even after operations on blastulae which lead to the development of abnormally small but otherwise complete early embryos, involving reduction in number of cells assigned to each somite. Evidence presented suggests that a body-position gradient may be involved, but in rather different ways at different stages, in controlling total somite number.

SPATIAL patterns of cell differentiation underlying morphogenesis in early embryos fall into two categories. One is the specification of non-repeating anatomical features such as heart, kidney, and limb and tail rudiments, henceforth referred to as the 'whole-body pattern'. The other is the specification of serially repeating units of morphogenesis, typified in vertebrate embryos by the somites, the segmental blocks of mesoderm from which serially repeated elements of axial skeleton and musculature develop. Development of whole-body and repeated patterns is coordinated in space, in that somite no. n in a particular species is quite reliably opposite, for instance, the hindlimb bud.

An elaboration of earlier gradient models, proposed by Wolpert¹, postulates a continuously graded cellular 'positional information' variable across tissue. Commitment of a cell to a given developmental pathway depends on its 'interpretation' of the value of the variable at a given point in the gradient. On such a model, although the spatial variable underlying pattern formation is simply distributed, the burden on the genetic machinery underlying cellular interpretation is correspondingly demanding, and the mechanism becomes implausible for serially repeated structures. This is because it is hard to see how the interpretative system within cells could be so organised as to react in a particular way, for example by deadhesion to form fissures between the successive, similar somite blocks, only to some thirty, regularly dispersed values on a gradient, although ignoring or reacting differently to intervening ones.

Models that postulate prepatterns²⁻³ in developing tissue have therefore been preferred. These are defined as the regular repetition in space of some special situation (for example a concentration peak of a 'morphogen' substance), each repetition causing development of one of the pattern elements subsequently seen in differentiation. The problem of interpretation by cells then becomes simplified, perhaps even to a binary choice controlled by a threshold concentration (for example, deadhesion as opposed to adhesion for somites, bristle-cell as opposed to epidermis for certain insect patterns). There are, however, other difficulties, connected with repeatability and consistency of the patterns in

space. In the case of somites, the observed constancy and regularity of element size and number⁶ is embarrassing for all known prepattern models^{7,8}.

Regulation is an even more profound problem. When overall cell number of early vertebrate embryos is reduced, cell numbers developing along each pathway are reduced to give a normally proportioned whole-body pattern. There are several plausible mechanisms for regulating the steepness of a continuous gradient to account for such regulations¹. The distance between 'peaks' in prepatterns underlying repeated structure, on current models^{3,4}, is determined, however, by physiochemical parameters (diffusion and allosteric interaction constants for specific molecules) which cannot easily be imagined as modifiable by changing the overall dimensions of the tissue. A prediction is therefore that the number of elements in a repeating pattern will be reduced from normal in direct proportion to amount of early embryonic tissue removed, if the latter amount corresponds to more than one whole unit's worth.

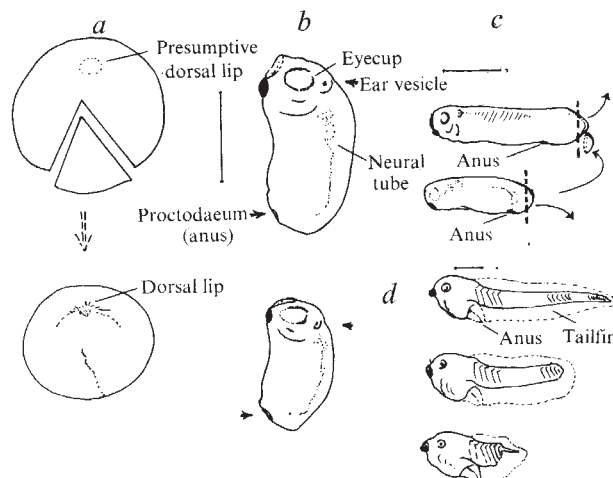


Fig. 1 *a*, Vegetal view of stage 8 blastula showing size reduction operation. This was performed in two-thirds strength Niu-Twitty solution¹³, but with the $\text{Ca}^{2+}\text{Mg}^{2+}(\text{Cl}^-)$ concentration doubled and pH brought to 7.0 with HCl. Healing was for 1 h in a fitting depression in wax, before storage to develop in one-tenth strength solution. Healed small early gastrula shown. In control siblings only one of the two cuts used to remove material was made, and healing allowed in the same solution. Normal repositioning of an excised section could not be achieved, and development of such animals was too disturbed for use as control material. *b*, Neurulae at stage 25, control and experimental, showing a size difference close to the maximum compatible with final development of experimental to the later larval stages. *c*, Early 40s stage larvae, control, and after tailbud tips removal at stage 24 and stage 26. Scale line represents approximately 1 mm throughout.

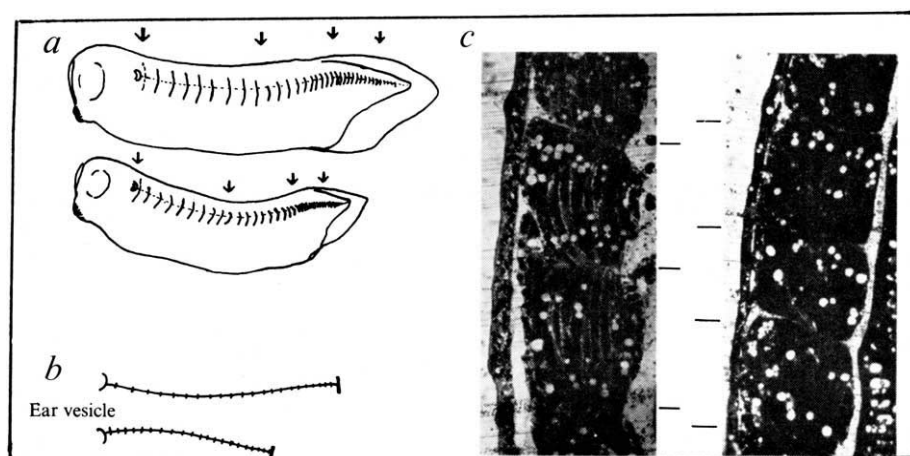


Fig. 2 *a*, Camera lucida drawing of an experimental and control pair at stage 34/35, each having 35 or 36 postotic somites, as seen after epidermis removal during fixation. Postotic axial length ratio now 0.83, having been 0.70 in stage 23 tailbud embryos. Arrows mark ear vesicle, 10th, 20th and 30th somites, seen to be appropriately placed. Somite boundaries are marked, but their outlines not drawn. *b*, Camera lucida record of somite boundaries in isolated axes between ear vesicle and level of proctodaeum. Length ratio best estimate is 0.75. Experimental has 21–22 somites, whilst its control has 23. *c*, Horizontal 1- μ m epon sections stained with toluidine blue, showing mid-trunk somites of an experimental and its control at stage 25/26. The shortness of the experimental somites can be seen. Marker lines are opposite inter-somite boundaries.

Regulations of somite number

Measurements of total length and of total somite number, in synchronously developing sibling *Xenopus* larvae, show no detectable correlation between the two. This suggests that despite variation in amount of egg material, total length variation in the somite-forming tissue is not an important source of variation in somite number. I have tested this conclusion further by removing animal-vegetal sectors from near the ventral meridian of stage 8 (ref. 9) blastulae (Fig. 1*a* and *b*) following which tailbud stage embryos are obtained of as little as five-eighths normal length. These animals have developed a complete whole-body pattern across their reduced number of cells. Cell counts per area in epidermal preparations show no evidence that extra mitoses have occurred by these stages in the experimental animals; they have regulated in the classic sense that the positional information theory¹ was evolved to explain.

I have counted and measured the numbers and sizes of post-ear-vesicle somites in operated embryos and their control siblings, fixed at the same time post fertilisation. Embryos down to little over two-thirds control length show essentially normal numbers and relative positions of somites at each stage (Fig. 2 and Table 1), revealing that the available prepattern models cannot account for somite formation.

Another class of mechanisms which can be clearly ruled out are those that would depend on particular, species-specific cell numbers. The length of individual somites seems to be determined by total length of tissue available, and numbers of cells per somite adjusted accordingly. Both within and between control embryos, cell numbers in the original lengths of somites are almost constant throughout the trunk region, but in haploid embryos, having more, smaller cells than normal, cell number per somite is correspondingly larger⁶. This is easy to check because true mitosis in *Xenopus* somites is very rare after the process of deadhesion and rotation of successive blocks of somite cells⁶. Cell numbers across somite widths in later embryos are thus records of the numbers between fissures at the time of somite formation. In my experiments, the smaller somites in fact contain fewer cells than normal in this dimension, which is the one determining how many somites are 'fitted in' to the whole-body pattern.

Still smaller embryos tend not to develop beyond mid-tailbud stages, and to have poorly defined somites that cannot be counted. This may be for mechanical reasons such as reduced stretching of the normal-sized cells, so that they are insufficiently spindle-shaped to perform rotation movements, but other explanations to do with the as yet obscure process of pattern formation itself are possible. One exceptional embryo, measuring only 0.6 of control length between ear vesicle and rear end, showed 4 clear rotated somites on

Table 1 Comparison of numbers and lengths of somites formed behind ear vesicle level, relative to length of axis traversed by the somite morphogenetic process, in experimental small embryos and synchronous control siblings

Sets of synchronous siblings operated at stage 8	Total mesodermal length behind ear vesicle as % of control length	Proportion (%) of distance from ear-vesicle-proctodaeum (anus) occupied by somites	No. of rotated somites posterior to ear vesicle	Thus, total length of mesoderm behind ear vesicle incorporated into somites, as % of control value	Length of equivalent set of 8 (3–10 post ear vesicle) somites (%)	No. of cells in width (original length) of some anterior trunk somites, averaged from counts on 4 separate sections per somite
Set I Controls 1	—	69	11–12	—	—	—
(stage 24+) 2	—	74	11–12	—	—	—
Experimentals 1	77	73	11–12	79	78	—
2	76	75	11–12	77	78	—
3	65	74	10–11	68	76	—
Set II Control	—	90	17–18	—	—	—
(stage 27) Experimental	80	90	16–17	79	81	—
Set III Control	—	100 (opp. anus)	20	—	—	9.5 10.0 9.5 9.75 10.0 10.5 10.25
(stage 29) Experimental	80	over 95	18	74	79	9.0 8.5 8.5 8.75 8.0 8.25 8.5
Set IV Control	—	100 (opp. anus)	21	—	—	—
(stage 29) Experimental	68	85–90	16	61	65	—
Somite morphogenesis extends to tailbud	—	% of total post e.v. mesoderm occupied by somites	—	Thus, total length of somitised mesoderm as % control	—	—
Set V Control	—	73	22–23	—	—	—
(stage 31) Experimental	85	76	20–21	89	72	—
Set VI Control	—	85	28	—	—	9.75 9.5 10.25 9.75 10.25 10.0 9.5
(stage 32) Experimental	90	88	26	92	81	7.5 7.0 8.0 7.5 7.75 8.0 6.75
Set VII Control	—	about 95	33+	—	—	—
(stage 34) Experimental	86	about 95	33+	86	84	—

Embryos were either prepared for wax or epon sectioning, or stripped immediately of epidermis for somite counting and measurement. Measurements were derived from constant scale camera lucida drawing, and cells in widths of mid-trunk somites were counted at notochord level in horizontal section⁶. Nuclear counts in epidermal whole mounts and sections give no evidence of smaller cells, as a result of compensatory mitosis since operations, by early tailbud stages. Sets consist each of experimental and synchronous control embryos, fixed at the same time after fertilisation.

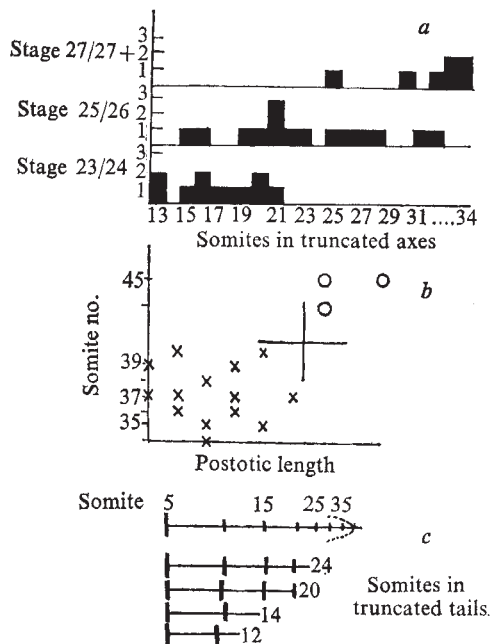


Fig. 3 *a*, Histogram of somite numbers in truncated axes of a population of siblings at stage 39, following tailbud tip removal at stages 23/24, 25/26 and 27/27+, respectively. Line represents average postotic somite number in synchronous control (unoperated) population. *b*, Scale chart of mean *camera lucida* positions of boundaries between successive groups of 5 postotic somites in the control population of *a*, in comparison with records, drawn in alignment, for four individual truncated axes.

one side, anteriorly, each 5–6 rather than the normal 9–10 cells in width (original length).

Yet another alternative model, in which appropriately proportioned blocks of cells are cut off by a local adaptation of the lengths of blocks to the width and depth of the column of presomite cells (the latter dimension regulating as part of the whole-body pattern after operations), can be ruled out. In the related amphibian *Bombina*, the morphogenesis of which is slower, I have been able to remove, at the close of gastrulation, a longitudinal strip of the sheet of cells that will later form the somite column in the trunk region, on one side only. Such animals develop with the column of presomite cells much shallower and narrower on the operated than on the normal side, over a distance equivalent to many somites. Somite numbers formed, however, prove to be equal on each side, in register, and normal for the species.

Somite number and growth controlled together during tail development

During mid-tailbud stages in amphibian embryos, when the above observations were made, the first true cellular growth, as opposed to cleavage, occurs especially in the tailbud region. Somite segmentation extends progressively into this, posteriorly, with rapid growth occurring at all levels not yet reached by onset of notochord and somite histogenesis. But increasing somite number remains in register with morphogenesis throughout growth in both control and experimental (initially small) animals, so segmentation must continuously be adapted to total length of tissue available.

The problem of coordination of growth and differentiation arises most acutely for structures such as limb and tail rudiments, in which the two processes are proceeding simultaneously. Summerbell *et al.*¹⁰ have suggested that in the developing chick limb, positional value of mesoderm cells, though they are dividing throughout the organ in true growth, changes with time only in a terminal un-

differentiated 'progress zone', where progressively more 'distal' values are achieved. Thus following successively later apical ectodermal ridge removals from wingbuds, a succession of more nearly complete, yet still distally truncated patterns were finally differentiated.

I have performed analogous experiments on *Xenopus* early tailbuds, and the results suggest that pattern formation is controlled in a similar manner. Following a suggestion of Graeme Mitchison, a terminal piece of undifferentiated tailtip mesoderm with ectoderm, always some 300 μ m long, has been removed from sibling embryos at a continuous range of tailbud stages, followed by counting of the total somites formed in the truncated tails of the advanced larvae 48 h later. Figure 3 shows typical results, from one of several such experiments consisting of 33 tailtip removals and 14 controls. Following tip removals between stages 23 and 27, that is, well before somite formation has reached the undifferentiated tip issue, the tails of the advanced larvae are sharply truncated, ending in short cone- or rod-shaped blastemas. They contain appropriate numbers of normal sized somites up to the levels of truncation (as measured by length), and the tailfin morphology, cumulative tail growth and lengths of successive groups of five somites are all literally parallel with controls up to the truncation point (see Figs 1c and 3c). The correlation between the proportion of a whole axis remaining, as judged by these criteria, and age of tip removal (Fig. 3a) is highly significant in spite of inevitable variation in the amount of tissue removed, particularly from very squat early tailbuds.

From Fig. 3a it seems likely that the somite numbers actually seen in truncated tails are a random sample from all possible integer numbers up to that in controls. When all the experimental populations are considered, no gaps in the series or traces of any stepwise distribution are seen. Taken together, these results indicate that position value of cells is fixed, as a continuous variable³ of some nature rather than in prepattern terms, very early during tailbud growth, and probably when individual cells are near the tip. Thereafter, it would be maintained with at most a local 'averaging' between neighbour (daughter?) cells.

Alternatively, the position values for an entire tail (that is for all the somite complement) could be present across the cells of a very young, squat tailbud, and thereafter maintained by clonal inheritance and averaging throughout growth. Then, removal of a uniform amount of tailtip from progressively older tailbuds would result in truncation of progressively less of the distal portion of a position gradient, and hence of somite pattern. This concept would however imply one of two novel features: either an initial gradient, at a time of setting up, that is much steeper posteriorly than in the trunk (implied by the large number of presumptive somite positions there in relatively little tissue) or else a changing relationship, posteriorly, between 'wavelength' of the somite forming process and gradient steepness. The former is possibly not readily allowed for by any of the models for maintaining gradients¹, but the second idea cannot be discounted at present. Indeed, the number of cells in the width (that is the length, on first formation) of somites does decrease in a regular way posteriorly (my unpublished data).

Idea of a locally inherited position value gradient

Amphibian mesodermal cells are probably undetermined with respect to whole-body position value (however this is encoded) only in two situations: while within the regulating positional information gradient that embraces trunk values and exists across the early embryo until around gastrulation (see the miniature whole embryo discussed earlier), or else while in a 'progress zone' of labile region near the tailbud tip, where the graded series of values is extended to complete the axis. Such a position gradient, if preserved by

local mechanisms among descendants of cells, would still be available to cause coordinated placement of later-developing structures (for example: limb buds, or the special behaviours of particular spinal nerves in the series¹¹).

The results discussed indicate that the steepness, or rate of change within embryonic tissue, of the positional variable is able to control the distance between repetitions of a certain cellular event in the animal's long axis. For reasons given at the outset, it is hard to imagine that this could be by direct interpretations of positional values as has been postulated for the whole-body pattern, but a theory of somite control, accounting for the known features of the process and its coordination with other morphogenesis, is to be presented elsewhere¹².

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Positional signalling and specification of digits in chick limb morphogenesis

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The interpretation of positional information can provide the basis for pattern formation in limb morphogenesis. The gradient in positional information along the antero-posterior axis, which is specified with respect to a localised boundary region, can be modified by grafting this region to successive positions along the axis. The pattern of digits obtained is consistent with a model based on diffusion of a labile morphogen and is thus similar to models proposed for the development of pattern in invertebrates.

RECENT approaches to pattern formation, that is the development of the spatial pattern of cellular differentiation, have made use of the concept of positional information¹. The suggestion is that cells are assigned positional information in relation to a coordinate system and the interpretation of this leads to appropriate cytodifferentiation. This is essentially an extension of earlier gradient models². Good evidence for positional information comes mainly from studies on the insect epidermis^{3,4}, insect eggs⁵ and regeneration in hydra^{6,7} but there are few demonstrated instances in vertebrates. We have suggested that the development of the pattern of the cartilaginous elements of the chick wing can be viewed in terms of the concept of positional information^{8,9}. The positional values assigned to the cells define a set of cell states, some of which will, for example, result in cartilage formation and others in the formation of muscle. We have proposed that the cells are assigned positional values along two of the axes, the proximo-distal and antero-posterior, by somewhat different mechanisms, the two positional values providing a two-dimensional coordinate system (Fig. 1). For the proximal-distal axis we have presented evidence that there is autonomous change of positional value with time in the progress zone^{8,10}. This is a region about 300 µm thick at the distal end which is specified by the apical ectodermal ridge (AER). Since all the cells in the progress zone are dividing, cells constantly leave the zone, those coming out later being assigned more distal positional values. This mechanism does not require signalling between the mesenchymal cells, although there may well be short range interactions, or local interactions involving averaging

of positional value. By contrast, for the antero-posterior axis, we suggested that the positional value is specified by a positional signal from the polarising region at the posterior edge of the progress zone^{9,11}. This was a conjecture based upon the work of Saunders^{12,13} and we now present evidence for a signal from the polarising region providing positional information for the specification of the digits in the chick wing. Crick¹⁴ has emphasised that diffusion could provide the basis for positional information and our data is consistent with such a mechanism.

The zone of polarising activity (ZPA) was discovered by Saunders and Gasseling¹². An additional ZPA grafted to a more anterior level in contact with the AER, resulted in the formation of extra skeletal elements. Of particular interest was the relationship of the additional elements with the normal ones; when between the host ZPA and the grafted ZPA, they were always in mirror image symmetry about the long axis. Moreover the polarity—the order of the induced digits—was such that there was a strong

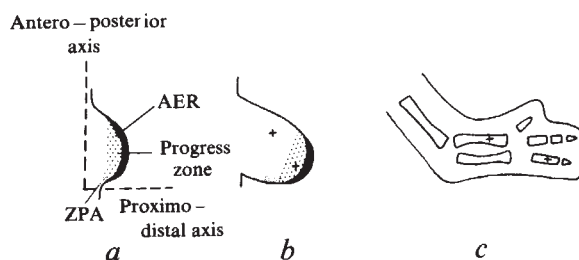


Fig. 1 The chick wing bud starts as a small bulge (a) and grows out as a tongue-shaped mass consisting of mesenchyme encased in ectoderm. The ectoderm has a thickening running along the distal rim, the apical ectodermal ridge (AER). The skeletal and muscular elements differentiate within the mesenchyme in a proximo-distal sequence (c). It is suggested that cells are assigned positional information when they are in the progress zone, along, for example, the antero-posterior and the proximo-distal axes. In the case of the antero-posterior axis, position is specified with respect to distance from the zone of polarising activity (ZPA). In b there are two points marked (+), the one outside the progress zone has already had its positional information specified, while the other is in the process of being specified. At a later stage, cells at these points interpret the positional information giving rise to tissues of the radius and digit 4 respectively (c).

Somite Number	Predicted effect of ZPA grafts at different levels									
14/15	Z									
15		Z								
15/16			Z							
16				Z						
16/17		2	3	4	Z	4	3	2		
17			2	3	4	Z	4	3	2	
17/18				2	3	4	Z	4	3	2
18	2	2	2	2	2	2	3	4	Z	4
18/19	3	3	3	3	3	3	3	3	4	Z
19	4	4	4	4	4	4	4	4	4	Z
19/20	z	z	z	z	z	z	z	z	z	z

Fig. 2 At the left is the outline of the normal limb bud and the somites at stage 19: the normal position of the ZPA (z) is also shown stippled. The predicted pattern of digits when an additional ZPA (z) is grafted to successive positions along the antero-posterior axis is shown in the columns on the right. It is based on the assumption that the digits are specified with respect to their distance to the nearest ZPA, that is a simple linear gradient. The predictions are only meant to show in diagrammatic form the overall pattern, and changes in limb geometry have not been allowed for. Nevertheless all the cases have been found experimentally except those enclosed by the solid line where either no digits were formed, or were much reduced. Those cases in which the digits are enclosed by a dashed line, are where digits formed but were sometimes inconsistent with the prediction, that is instead of 43, either 432 or 32 was obtained.

tendency for posterior structures to be adjacent to the graft¹³. We have interpreted these results as implying that the ZPA is the boundary or reference region for the antero-posterior axis of positional information^{9,11}. Since only cells in the progress zone can respond to the influence of the ZPA¹⁵ the character of the digits may be specified by their distance from the ZPA at the time when they are leaving the progress zone. One way in which a gradient in positional value could be set up would be by the ZPA being the source of a diffusible morphogen which would act as a positional signal.

In the first instance we will consider a model which is not dependent on any specific assumptions with respect to mechanism, such as diffusion, and only assumes that positional information is specified by the distance from the

nearest ZPA; that is we assume a simple linear gradient. The best markers along the antero-posterior axis are the three digits (II), (III) and (IV) (which for convenience we will represent as 2, 3, 4) since they can usually be recognised unequivocally by the number of elements and distinct morphological characteristics. We will thus confine our attention to them and assume that the nature of the digit specified is determined by its distance from the nearest ZPA. We have shown diagrammatically the predicted pattern of digits when an additional ZPA is placed in successive positions along the antero-posterior axis, the host's ZPA being left intact (Fig. 2). From this several interesting features emerge. First, digit 2 will form wherever the gradient has an appropriately low level and thus it should be possible to obtain an additional digit 2 on its own when the ZPA is placed in a sufficiently anterior position. Second, one does not get discontinuities such as digit 2 next to digit 4. Third, there is no requirement for intrinsic polarity, that is the signal from the ZPA propagates in both directions.

In the normal limb the digits form mainly in the posterior half of the limb, that is from the region between somites

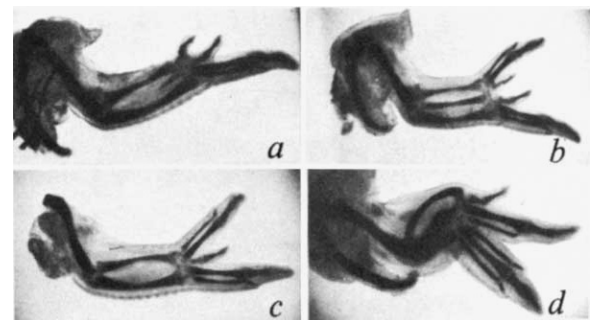


Fig. 3 A series of dorsal views of whole mounts of right limbs with grafts showing the pattern of digits formed as the grafted ZPA is placed in successive posterior positions. The digits formed are as follows from anterior to posterior; a, 2234; b, 432234; c, 43234 and d, 234434.

18 and 19¹⁶. The ZPA occupies a position immediately posterior to this (Fig. 2). The anterior half of the limb does not seem to participate in formation of distal structures and this may be related to the asymmetry of the AER which is better developed posteriorly.

Grafting the ZPA

We have substantially confirmed all the predictions by grafting ZPA tissue to successive positions along the antero-posterior axis. ZPAs were taken from stages 18 to 21 and pinned with a small piece of platinum wire into a hole cut into the edge of the limb¹³. The graft was thus always in contact with the AER except when placed in more anterior positions. We have made use of the somites as markers for position along the antero-posterior axis. Since the distance between somite centres is about 250 μ m and the size of the ZPA graft is about the same, the error in the positioning of the graft is about half the width of a somite. The results are substantially the same for stage 18 and 20 hosts (Table 1).

Considering structures posterior to the graft first, it can be seen that when the graft was opposite somite 15 which appears to be just outside the anterior border of the limb bud, normal limbs developed in most cases. This confirms that the ZPA from the wing does not itself differentiate into any recognisable structure¹⁵. This suggests that the signal from the ZPA is rather short range and that body wall tissue is not competent to respond to the signal. Of particular importance is the development of hands comprising 2234 (Fig. 3a) and 32234 when the graft is in the

Table 1 Formation of digits between the grafted ZPA and the host ZPA when the ZPA is placed in successive positions along the antero-posterior axis

Stage 18	Position of graft with respect to somites									
Digits	14/15	15	15/16	16	16/17	17	17/18	18	18/19	19
234		6								
2234		1								
32234		1	3							
432234			4	3						
43234			3	5						
4334					4	5	2	1		
434						1	4	4	1	
44										
4								1	2	
None									2	4
Stage 20										
234	7	3	2	2						
2234		1	2		1					
32234		1		1						
432234		1	2	2	1					
43234		1		3	2					
4334					3	4*				
434						1	5*			
44										
4								2		
None							2	8	11	2
									2	1

The data refer to the number of cases obtained.

* In some of these cases the identification of the anterior digit 4 was equivocal.

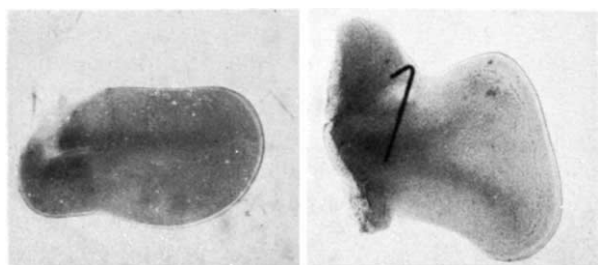


Fig. 4 Dorsal view of whole mounts of the normal and grafted right limbs at stage 24. The ZPA was placed opposite somite 16 at stage 18 and the width of the limb has increased by 50%. Note the pin which held in the grafted ZPA.

region of somites 15 and 16. This shows that the ZPA can specify either digit 2 or digits 23 without formation of digit 4, and thus precludes any mechanism for specification of the digits based on sequential induction. On the contrary this result is consistent with a graded signal which specifies digit 2 when the lower part of the gradient first begins to lie within competent limb tissue. It is of interest that this result also suggests that tissues can transmit the signal without responding to it. Similarly, in a variety of other grafts where we had reason to believe that the signal from the ZPA has been attenuated by, for example, the presence of a Nucleopore or Millipore filter, it is often digit 2 only that is specified.

As the graft is placed in successively posterior positions, digit 4 develops anteriorly and a complete mirror image duplication occurs (Fig. 3b); and when placed still more posteriorly, first one digit 2 and then the other, is no longer formed between the two grafts (Fig. 3c). When the graft is opposite somites 17 to 18 only a 4334 or 434 forms posterior to the graft. When the graft is, however, moved slightly more to the posterior, contrary to expectation, no 44 combinations could ever be identified with certainty. On occasion, a small single 4 could be found. When the graft was placed opposite somites 18 and 19 at stage 18, which is quite close to the host's ZPA, normal limbs were usually formed, the digits being anterior to the graft. This suggests that the signal from the ZPA is not affected when two ZPAs are placed together. But, at stage 20 this graft often resulted in the complete absence of digit 4.

The structures formed anterior to the graft were more variable. Nevertheless, they conform quite well to the predictions of Fig. 2. Digits anterior to the graft usually only formed when the ZPA was placed opposite somite 17 or more posterior. The most common results observed were 23 and 234. At stage 18 some grafts resulted in 34 and single digits, possibly digit 4. Where a complete anterior hand 234, was present, the posterior hand was always 434 or less (Fig. 3d). One exceptional case produced 2344334, the largest number of digits we ever obtained along the same antero-posterior axis.

The results as a whole conform well with both the general and specific predictions of Fig. 2. The main deviations occurred when a single or double digit 4 were predicted in isolation. In this connection we have some evidence that a digit 4 begins to develop but fails to do so because of degeneration of the associated vascular system.

As Saunders¹³ has pointed out, the grafted ZPA can bring about changes in the extent of the AER and thus the outgrowth of the limb. In fact, placing the ZPA in a variety of positions near the tip of the limb is without effect unless it is adjacent to the AER. In the normal limb the outgrowth is not symmetrical about the long axis (Fig. 4). By contrast when a ZPA is placed anteriorly (level somite 16) the outgrowth is symmetrical about the long axis, the limb increasing in width. Measurements of the change in width in operated limbs show that the increase in width seems to

correlate with the increase in number of digits obtained. From the point of view of digit specification the crucial width is that at stage 24 when the digits begin to be laid down¹⁷. For example, Fig. 4 shows the difference at stage 24 between a normal limb and one which had a ZPA grafted opposite somite 16. There is a 50% increase in width. It should be noted that there does seem to be a constraint on the number of digits than can be specified: this is very rarely greater than six.

Diffusion model for the signal

The predictions of Fig. 2 are based on a simple linear gradient and the question now arises whether this gradient could be provided by a diffusible morphogen. In general terms, diffusion would seem to provide a suitable mechanism particularly since the signal from the ZPA is propagated in both directions, and no discontinuities in level were found. Moreover the time/distance relationships are compatible with diffusion since the gradient is set up over about 500–1,000 μm and the time available is of the order of 10 h. It is perfectly reasonable to set up a diffusion gradient over this distance in the available time¹⁴.

It is possible to set a linear diffusion gradient with a source and a sink at the ends¹⁴ but in the chick wing, although the ZPA can be considered as a source, there does not seem to be any region corresponding to a localised sink. Thus it is necessary to assume the morphogen to be broken down and this will give a gradient with an exponential form. In this case the concentration of the morphogen at a given distance from the ZPA will not be constant, but will vary according to the presence of the second ZPA and this is inconsistent with Fig. 1. Ignoring the complex geometry of the limb, consider the simple one-dimensional case in which the ZPA would be the source of a morphogen which is broken down; the concentration at the ZPA being fixed (Fig. 5). If another source is now placed at the other end, analogous to placing another ZPA at the anterior border of the limb, the resulting concentration of the morphogen is as shown in Fig. 5, the concentration in the central region being considerably increased. Such a distribution is inconsistent with the results, for on any

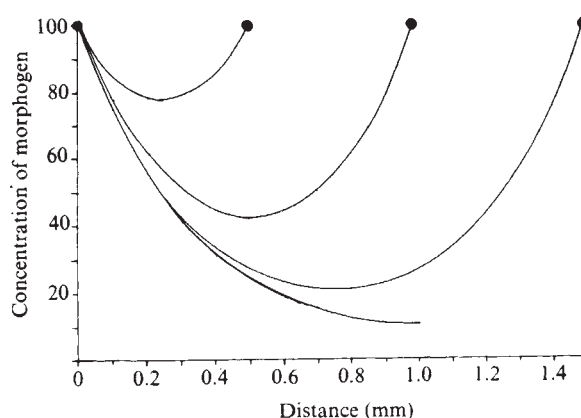


Fig. 5 The concentration profile of a morphogen for the one-dimensional case, which is taken to represent the antero-posterior axis. The source (●) of the morphogen, representing a ZPA, is fixed at 100 and the diffusion constant and rate of breakdown were chosen such that, for the case of a single source, the value 1 mm away from the source would be 10. The diagram also shows the profiles of the morphogen when a second source is added at varying distances along the axis. The concentration in the middle region is that most affected and is no longer that corresponding to the case of the single source. For example, when the two sources are 1 mm apart, the region 0.5 mm from the ends has its concentration increased from 24 to 42. If the distance between sources is increased to 1.5 mm, however, the concentration 0.5 mm from the end is relatively unaffected.

plausible values of the concentration for specifying the digits, a graft opposite somite 16 should result in the absence of digit 2 in the middle region which is not the observed finding. But we have so far ignored the fact that when a ZPA is grafted to an anterior position the width of the limb bud does not remain constant, but increases substantially. If there is an increase in width of the distal region of the limb, the profile of the morphogen will be significantly altered (Fig. 5) since the sources are now further apart. For the highly idealised case, an increase of 50% would give a profile compatible with the results. More complex models, taking into account a homeostatic component³ would also tend to reduce the concentration in the central region, but if the increase in width had not occurred it would have made a diffusion model very unlikely. There is in fact some evidence that cells maintain their positional value when the signal is removed, for the total removal of the ZPA and the region that will normally give rise to digit 4 leads to the loss of digit 4, but digits 2 and 3 are normal.

Our results strongly suggest that the digits in the chick wing are specified by a gradient in positional information, the nature of the digit being determined by its distance from the ZPA. The results are consistent with the specification of positional value by diffusion of a labile morphogen from the ZPA, which may be considered as a boundary region. This implies that the character of the digit is specified by a concentration gradient acting as a positional signal, but we do not know how this is read off by the cells. It should be noted that we have assumed specification of the digits to occur at the same time: it is possible to have a model in which they are specified by a series of sequential decisions (J. Lewis, personal communication). The ZPA also seems to affect the outgrowth of the limb, the width being significantly increased. This may be mediated by the

AER and the ZPA has little effect if not in contact with it.

Although we have concentrated only on digit specification here this gradient would provide the positional information along the antero-posterior axis for all elements of the limb and it is clear that the ZPA does have an effect on the proximal elements such as humerus, radius and ulna^{11,14}. Moreover, it is known that a similar system is operative in the leg and the ZPA of wing and leg are interchangeable¹⁵. It thus shows that in the chick limb, pattern formation can occur in a way strikingly similar to that found in some invertebrates, and strengthens the view that there may be universal mechanisms. Similarities to the classical organiser may also be noted.

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letters to nature

Local interstellar medium

NEUTRAL interstellar hydrogen can be prevented from penetrating freely into the heliosphere of solar plasma, because it is partially coupled to the subsonic flow of interstellar plasma. Results from modelling the backscattering of the diffuse Lyman- α radiation are therefore unreliable. The interstellar density of H^+ is probably 0.02 cm^{-3} or less, and the density of neutral hydrogen greater than 0.2 cm^{-3} .

Present thinking favours the idea that the interstellar medium in the neighbourhood of the Solar System is low density "intercloud" gas. With heating by postulated MeV cosmic rays or soft X rays, a stable state could exist¹ with atomic hydrogen density $N \simeq 0.1 \text{ cm}^{-3}$, temperature $T \simeq 8,000 \text{ K}$ and an ionised component of H^+ and e^- of density $n \simeq 0.03 \text{ cm}^{-3}$. The plasma density might be larger because of the ionising ultraviolet emission from local hot stars².

Observations of Lyman- α absorption in the spectra of nearby stars indicate^{3,4} generally higher values of the mean gas density $N \simeq 0.2-0.6 \text{ cm}^{-3}$. The interpretation of the all-sky maps of the diffuse Lyman- α backscatter is still the subject of debate: the various models give⁵⁻⁷ densities $N_L \simeq 0.05-0.25 \text{ cm}^{-3}$, and streaming velocity relative to the Solar System $V = 10-20 \text{ km s}^{-1}$, but are inconclusive about the temperature. Both sets of data imply that the local medium would be the low density, intercloud state, being far from the typical density 10 cm^{-3} of

clouds¹. But if, as I argue, the density N_L within the Solar System is significantly lower than the undisturbed gas density N , it seems that the local medium is not particularly close to the theoretical intercloud state.

My argument is based on the global picture of gas and plasma flow in the neighbourhood of the heliosphere (Fig. 1). Two plasma shocks are shown, one terminating the supersonic solar wind and the second a bow shock^{8,9}, while a contact surface separates the interstellar plasma from the heliospheric plasma originating from the Sun (which acts as a source). The bow shock is a fast hydromagnetic shock and exists with the plasma parameters given earlier only if the magnetic field is rather weak: for $B < 10^{-6}$ gauss, the magnetosonic Mach number $M = V/[(B^2/\mu_0 nm) + (2\gamma kT/m)]^{1/2}$ can exceed unity. The plasma behaves as a fluid on a small, ion gyro-radius scale, while the neutral gas moves almost freely through it. But, in practice, the gas must interact with the plasma on the large heliosphere length scale, effecting frictional drag and cooling, and so distorts the flow from the standard one of a supersonic source in a supersonic stream. Indeed, the terminating shock of the solar wind is probably weak or even non-existent¹⁰ because the plasma-gas interactions effect deceleration over an extended region, analogous to the expansion of ordinary gas into a rarefied background medium¹¹. The plasma-gas interactions dominate if $B/N_L \gtrsim 10^{-5}$ gauss cm^3 , in which case the heliosphere radius is¹⁰ $R \simeq 60 N_L^{-1} \text{ AU}$ (where N_L is in cm^{-3}), about twice the sonic position's mean radial distance.

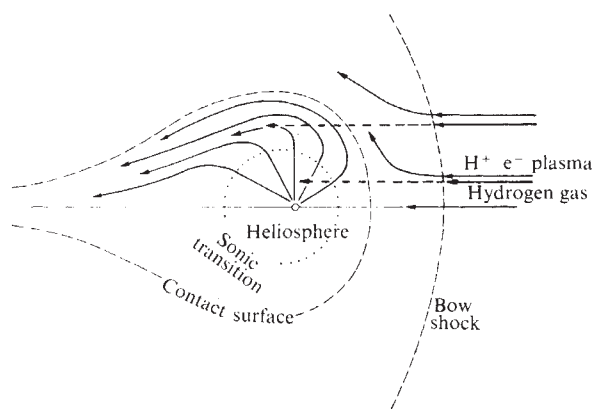


Fig. 1 The structure of the magnetised plasma flow in the neighbourhood of the heliosphere. The streaming hydrogen gas is partially coupled to the plasma, its direct component being depleted in penetrating the diverted and shocked interstellar plasma.

If the plasma-gas interactions were negligible the bow shock would stand off ahead of the heliosphere by a distance Δ , given roughly by

$$\Delta/R \simeq 1.1n/n' \quad (1)$$

where n' is the density downstream of a normal plane shock

$$n'/n = (\gamma + 1)/(\gamma - 1 + 2M^2) \quad (2)$$

Expression (1) is applicable^{1,2} in a wide range of values of γ and M , and even for weak shocks (see section 4.4 and Fig. 6.27 of ref. 13) with n'/n as low as 1.1.

The main plasma-gas interaction is the resonant $H^+ - H$ charge exchange process with a cross section at 1–3 eV ($\frac{1}{2}mv^2$ or kT) of $\sigma = 6 \times 10^{-15} \text{ cm}^2$. I can thus estimate the proportion of streaming hydrogen atoms which pass through the stagnation region into the heliosphere as $N_L/N = \exp(-\lambda)$, where

$$\lambda \simeq (1 + \nu)^{\frac{1}{2}} \Delta \sigma n' \simeq 1.1 (1 + \nu)^{\frac{1}{2}} R \sigma n \simeq 8n/N_L \quad (3)$$

The factor involving ν represents the increased collision rate in thermal plasma¹⁰ and is adequately approximated by taking $\nu = 0.7$ –1.0 in the present situation.

With the values n and N_L given above, the incoming hydrogen gas is appreciably depleted, by a factor exceeding three, and

$$N \simeq N_L \exp(8n/N_L) \quad (4)$$

exceeds 0.65 cm^{-3} .

This estimate is of course, very crude. Particles contributing to N_L can approach laterally, avoiding the highest density stagnation region, but then pass through a larger depth of subsonic plasma; also friction of the neutral gas causes expansion of the stagnation layer and increases Δ above the value (1); and a proportion of the neutralised protons penetrate into the heliosphere and contribute to N_L . Additional losses caused by interaction with the heliospheric subsonic plasma have been ignored (this latter is much hotter¹⁰ at O (100 eV), so is less dense by a factor 100 and has little effect). But these considerations are to some extent compensatory and do not seem important enough to change the estimate (3).

Another possibility is that the interstellar field B is stronger, so that the plasma flow is sub-Alfvénic and the bow shock absent. It is then necessary to calculate

$$\lambda = \int [1 + \nu(s)]^{\frac{1}{2}} n'(s) \sigma(s) ds$$

through the upstream flow, with σ larger for the smaller relative velocities and with some outer cutoff. The frictional drag of the gas would distort the flow more severely, but otherwise the result for λ should not be much different from equation (3). The principal change with a larger B would probably be a decreased heliosphere radius R , but by only a factor of two for a field as strong as 5×10^{-6} gauss (ref. 10).

There was an early suggestion¹⁴ that charge exchange interactions of shocked solar wind protons with hydrogen atoms of interstellar origin could contribute to N_L and to the diffuse Lyman- α backscatter. In a curious sense, I have here reached a contrary conclusion: that charge exchange processes impede the penetration of gas particles through the interstellar plasma into the solar system and reduce the local density N_L . The interstellar gas is indeed not only depleted, but its thermal and streaming velocities must also be changed through the substitution of some neutralised protons. Thus, analysis of the Lyman- α backscatter can tell us little directly about the real state of the local interstellar gas. With the crude correction (4), the real gas density is compatible with the Lyman- α absorption values^{3,4} only if the plasma density is $n \simeq 0.01$ – 0.02 cm^{-3} . It indicates that the local interstellar gas has somewhat higher neutral-hydrogen density and lower ion density than the standard theoretical values¹.

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Charged dust grains and excitation of rotational levels of interstellar molecular hydrogen

THE strengths of ultraviolet absorption lines of interstellar hydrogen have been measured in the spectra of several reddened early-type stars^{1–3}. Interstellar H_2 molecules are observed mainly in the two lowest rotational levels $J = 0$ (*para*- H_2) and $J = 1$ (*ortho*- H_2) with a ratio of populations corresponding to an average temperature $\sim 80 \text{ K}$. This temperature is consistent with the mean HI kinetic temperature determined in 21-cm absorption studies^{4,5}, whereas higher rotational levels up to $J = 5$ or 6 are populated corresponding to significantly higher excitation temperatures^{3,6} in the range 150–200 K. These observations indicate that a process other than thermal gas collisions is responsible for populating the higher rotational levels. The precise nature of the excitation mechanism is as yet unknown. A process which could contribute to non-thermal excitation of rotational levels involves encounters of charged dust grains with interstellar hydrogen. We have examined this mechanism and find that it could be of comparable, if not greater, importance in relation to other processes which have been proposed.

Since most interstellar dust particles are believed to originate in the atmospheres of cool Carbon or Mira-type stars, the velocity of their injection into interstellar clouds is $\sim 10^8 \text{ cm s}^{-1}$ (ref. 7). The velocity spectrum of interstellar grains resulting from the slowing of such fast grains due to gas collisions in clouds is⁷

$$f(v)dv = A/v^2, \quad v_{\max} \geq v \geq v_{\min} \quad (1)$$

where A is a constant, $v_{\max} \simeq 10^8 \text{ cm s}^{-1}$ and $f(v)dv$ denotes the number density of grains in the velocity range $v, v + dv$. The

minimum velocity $v_{\min}$ is significantly greater than the thermal speed determined solely by thermal gas collisions. Systematic dust-gas drift velocities are maintained in most regions of the Galaxy by the action of radiation pressure from integrated starlight^{8,9}. The total stellar radiation from the direction of the galactic bulge drives grains relative to gas at speeds $v \geq 10^5 \text{ cm s}^{-1}$ in a cloud with $n_{\text{H}} \approx 10 \text{ m}^{-3}$ and $T \approx 100 \text{ K}$ in the solar vicinity⁹. In the following discussion we assume $v_{\min} \approx 10^5 \text{ cm s}^{-1}$ to be the minimum velocity of a typical interstellar grain. The use of the distribution function (1) is strictly justified, if the slowing time of fast grains in the cloud is comparable to or greater than the residence time of grains within the cloud. For a cloud of dimension $\sim 3 \text{ pc}$, the dispersal time for grains with $v = v_{\min} \approx 10^5 \text{ cm s}^{-1}$ is $\sim 3 \times 10^6 \text{ yr}$. This is an order of magnitude greater than the characteristic stopping time of fast grains⁷. Thus the concentration of suprathermal grains may have to be diluted by a factor ten below that of all grains. But since charged grain acceleration mechanisms could operate in the interstellar medium the precise velocity distribution of grains remains uncertain and a larger proportion of grains may be suprathermal. There is also the possibility that at least some of the clouds for which H_2 observations are available, lie in the vicinity of stellar sources of interstellar dust, so that a high concentration of suprathermal dust grains could exist. In view of these uncertainties we tentatively adopt the simple distribution function (1) in the ensuing discussion. The mean velocity of an interstellar grain is then

$$\langle v \rangle = \frac{\int_{v_{\min}}^{v_{\max}} v f(v) dv}{\int_{v_{\min}}^{v_{\max}} f(v) dv} = v_{\min} [1 - v_{\min}/v_{\max}]^{-1} \ln[v_{\max}/v_{\min}] \quad (2)$$

$$\approx 6.9 \times 10^5 \text{ cm s}^{-1}$$

The role of the high energy tail of this velocity spectrum in contributing to the heating and ionisation of the interstellar medium has already been discussed^{7,10}. We confine our attention here to the behaviour of grains with velocities near to the estimated "average" speed $\sim 6.9 \times 10^5 \text{ cm s}^{-1}$. Such grains carry a net electric charge which corresponds to an electrostatic potential $\phi \approx 0.1 \text{ V}$ – 1 V (ref. 11); and collisions of these charged grains with H_2 molecules are quasielastic with an effective cross section

$$\sigma = \pi(fa)^2 \quad (3)$$

where a is a grain radius and $f \geq 1$. Although the precise value of f in equation (3) is uncertain, it may be expected to exceed one by a significant factor. This could arise from long-range electrostatic interaction of spherical particles as well as from the effect of surface irregularities and protrusions, such as whiskers, that may grow on grains which are in a state of rapid rotation. On the basis of cross sectional data for charged radical-neutral atom interactions¹² and other considerations it seems reasonable to set $f^2 = 10$.

The energy transfer to an H_2 molecule in an elastic encounter with a grain of velocity v is

$$\Delta E \approx m_{\text{H}_2} v^2 \approx 0.021 (v/10^5 \text{ cm s}^{-1})^2 \text{ eV} \quad (4)$$

where m_{H_2} is the mass of a hydrogen molecule. Setting $v = \langle v \rangle = 6.9 \times 10^5 \text{ cm s}^{-1}$ in equation (4) we obtain $\Delta E \approx 1 \text{ eV}$. The excitation energy of the J^{th} rotational level is

$$E_J = 0.0073 J(J+1) \text{ eV} \quad (5)$$

giving $E_J = 0.31 \text{ eV}$ for the highest observed level ($J = 6$). This energy is less than that transferred to an hydrogen molecule by a grain colliding with the "average" velocity given from equation (2). Charged dust grain- H_2 collisions must therefore

warrant serious consideration as a candidate for the excitation of rotational levels of interstellar H_2 .

The rate coefficients for the excitation of rotational levels by this process may be readily calculated. For excitation of level J the rate coefficient is

$$R_J \approx \pi f^2 a^2 \frac{\int_{v_J}^{v_{\max}} v f(v) dv}{\int_{v_{\min}}^{v_{\max}} f(v) dv} [n_{\text{g}}/n_{\text{H}}] n_{\text{H}} \text{ s}^{-1} \quad (6)$$

where n_{H} , n_{g} are respectively the number densities of hydrogen and of grains, and v_J is defined by equating ΔE given by equation (4) to E_J given by equation (5). This procedure yields

$$R_J = \pi f^2 a^2 \xi [n_{\text{g}}/n_{\text{H}}] n_{\text{H}} \text{ s}^{-1} \quad (7)$$

with

$$\xi \approx 7.5 \times 10^5 (1 - 0.067 \ln[J(J+1)]) \text{ cm s}^{-1} \quad (8)$$

Assuming a mass fraction ~ 0.02 of interstellar material in the cloud exists in the form of spherical suprathermal dust particles of density $s \approx 1 \text{ g cm}^{-3}$ and radii a we have

$$0.02 = (n_{\text{g}} 4/3 [\pi a^3 s]) / (n_{\text{H}} m_{\text{H}}) \quad (9)$$

which may be re-expressed

$$\pi a^2 (n_{\text{g}}/n_{\text{H}}) \approx 2.51 \times 10^{-21} (a/10^{-5} \text{ cm})^{-1} \text{ cm}^2$$

Using equations (7), (8) and (10) together with $f^2 \approx 10$ we obtain the results of Table 1.

Table 1 Values of R_J^*

J	$R_J (a/10^{-5} \text{ cm}) / n_{\text{H}} (\text{cm}^3 \text{ s}^{-1})$
1	1.80×10^{-14}
2	1.66×10^{-14}
3	1.57×10^{-14}
4	1.51×10^{-14}
5	1.46×10^{-14}
6	1.42×10^{-14}

*Since all the rate coefficients vary as $\sim a^{-1}$, grains of radii 10^{-6} cm , if they exist in comparable mass (as is suggested by ultraviolet extinction data), would dominate the excitation processes in the present theory.

For grains of radii $a = 10^{-5} \text{ cm}$ the rate coefficients calculated here are of comparable if not greater magnitude than those obtained for ultraviolet pumping (ref. 13 and unpublished work by S. P. T. and P. Joshi) for $n_{\text{H}} \geq 10 \text{ cm}^{-3}$. We also note that the excitation rate of the $J = 1$ level produced by the present mechanism is greater than the *para-ortho* conversion rate produced by proton encounters¹⁴ for a H^+/H ratio less than 10^{-4} .

In the preceding discussion it has been assumed: (a) that most of the transferable energy in the encounter between an H_2 molecule and a grain is converted into the rotational energy; (b) that the excitation cross section is equal to the collisional cross section; and (c) that the excitation cross section is independent of the relative energy of the collision. From classical considerations (a) and (b) are likely to hold true to a good approximation for quasielastic encounters between a "dumb-bell type" diatomic molecule and a solid surface. Experimental data on encounters between diatomic molecules and solid surfaces support these statements¹⁵. Assumption (c) is difficult to justify and could be valid only in a crude approximation. Since the precise population distribution among the various rotational levels depends strongly on the validity of this assumption a

more detailed quantum mechanical determination of the energy dependence of the excitation cross section is worthy of pursuit.

The depopulation of levels with $J \geq 2$ occurs mainly by spontaneous emission, the rates of which are greater than 10^{-11} s^{-1} (ref. 14). We note that this rate is much larger than the de-excitation rate of $1.73 \times 10^{-14} \text{ s}^{-1}$ by grain collision for $f^2 n_H = 10$, $a = 10^{-5} \text{ cm}$ and $\langle v \rangle = 6.9 \times 10^5 \text{ cm s}^{-1}$. Therefore, the population N_J of any level $J \geq 2$ may be assumed to be given by the excitation by collision of H_2 ($J = 0$) with grains followed by spontaneous decay. The population of the $J = 1$ level may be governed only by grain collisions. The values of N_J/N_{0gJ} so obtained using the spontaneous emission rates from Dalgarno and Wright¹⁶ are given in Table 2 for different values of J .

Table 2 Comparison of observed N_J/N_{0gJ} ratios with theoretical prediction

J	ζ Oph	Observed N_J/N_{0gJ}			Theoretical (N_J/N_{0gJ})
0	1	ϵ Per	ρ Pup	1	1
1	6.32×10^{-2}	1.12×10^{-1}	7.07×10^{-1}		1.16×10^{-1}
2	2.52×10^{-3}	3.97×10^{-4}	7.94×10^{-1}		1.13×10^{-4}
3	2.24×10^{-5}	8.92×10^{-6}	3.16×10^{-1}		1.57×10^{-6}
4	1.79×10^{-6}	2.24×10^{-7}	2.24×10^{-1}		6.08×10^{-7}
5	6.32×10^{-8}	8.92×10^{-9}	1.12×10^{-1}		4.49×10^{-8}
6	1.13×10^{-8}	—	—		4.13×10^{-8}

The observed values of N_J/N_{0gJ} for three stars⁶ have also been included for comparison. Table 2 shows that the agreement between the calculated and observed values of N_J is not unsatisfactory for the stars ζ Oph and ϵ Per. For ρ Pup, no agreement is possible and excitation of the rotational levels of H_2 has to be by process other than excitation by collisions with grains.

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Cosmological absorption of gravitational waves

SEVERAL people have investigated the absorption of electromagnetic and neutrino waves in cosmological models and discussed the self-consistency or otherwise, in the Wheeler-Feynman absorber theory of radiation, of retarded and advanced fields; a solution of the field equations is self-consistent if the absorption tends to completeness as the field propagates. Cosmological absorption of gravitational waves has been treated briefly by Hawking¹. Here, the completeness or otherwise of the absorption of retarded and advanced gravitational waves will be investigated using a technique introduced, in the case of electromagnetic absorption, by Davies²; the technique applies to the Robertson-Walker cosmologies of arbitrary spatial curvature.

In a cosmological model with scale factor R , consider a wave that has angular frequency ω relative to a comoving

observer; $\omega = \omega_i/R$, a subscript i denoting the value of a quantity near the source of the wave. Let t' be a time coordinate for which the metric is proportional to that of special relativity. Take a time factor $e^{i\omega t'}$ and let $\epsilon = -1$ and $+1$ for retarded and advanced fields respectively. Provided the refractive index does not vary too rapidly with the radial coordinate r , the absorption factor is

$$\exp[(\epsilon\omega_i/c) \int k dr] \quad (1)$$

where c is the speed of light in free space, k is the imaginary part of the refractive index and the integration is over the path of the wave. For fields propagating on light cones $dr = -\epsilon c dt'$ and for cosmological models that are spatially flat t' is related to the cosmic time t by $dt/dt' = R$, so that expression (1) becomes

$$\exp(-\int \omega k dt) \quad (2)$$

For gravitational waves, this factor is¹

$$\exp[-(G/2c^2) \int \eta dr] \quad (3)$$

where G is the Newtonian gravitational constant and η is the coefficient of viscosity of the medium.

For atomic hydrogen $\eta \sim 10^{-4} T^{1/2} \text{ g cm}^{-1} \text{ s}^{-1}$ where T is the absolute temperature. For fully ionised hydrogen in the absence of a magnetic field³ $\eta \sim 10^{-16} T^{5/2} \text{ g cm}^{-1} \text{ s}^{-1}$.

Consider absorption in a static homogeneous universe. The factor (1), with $k = G\eta/2c^2\omega$, shows that appreciable absorption will occur for propagation over a range d of r where $d \approx c^3/G\eta$. If H denotes the Hubble constant of the actual Universe, then a characteristic distance in cosmology is c/H , which is about 10^{28} cm . If $d \gtrsim 10^{28} \text{ cm}$, then the static model is inadequate to treat cosmological absorption. In the actual Universe, observations have shown that the intergalactic medium could consist of fully ionised hydrogen with⁴ $6 \times 10^8 \text{ K} > T > 10^4 \text{ K}$, corresponding in the static model to $d \sim 10^{23} - 10^{45} \text{ cm}$.

Suppose that all matter in the universe can be represented by a smoothed number density N . Consider cosmological models of Class I in which there is continuous creation of matter at such a rate as to keep N constant, and models of Class II, in which matter is conserved so that $N \propto R^{-3}$. As an advanced wave propagates into the past its wavelength decreases and, for Class I models, eventually becomes comparable with the mean free path of the particles of the cosmic medium; then the above formulae for the viscosity are inapplicable. Those formulae also fail eventually for retarded waves in Class II models, since the mean free path increases more rapidly than the wavelength. When the mean free path greatly exceeds the wavelength, so that the particle collision frequency ν is much less than the wave frequency, for a non-relativistic medium⁵ $k \sim N\nu T/\omega^3$.

Let σ_ϵ denote an effective cross section equal to $\omega k/Nc$. Absorption will be complete for retarded and advanced fields in the Robertson-Walker cosmologies if²

$$\left| \int^\infty N \sigma_\epsilon dt \right| = \infty \quad (4)$$

and

$$\left| \int^0 N \sigma_\epsilon dt \right| = \infty \quad (5)$$

respectively. For gravitational waves expressions (3), (4) and (5) show that $N\sigma_\epsilon = G\eta/2c^2$. Take $R \propto t^n$ with $n > 0$ and consider retarded gravitational waves. In Class I universes, if the temperature of the cosmic medium is constant, then expressions (3) shows that absorption is complete for all n . In Class

II universes, take $k \sim NvT/\omega^3$. Also, take $v \sim NT^\alpha$ where α is a constant; thus $N\sigma_e \sim N^2T^{\alpha+1}/\omega^3$. Suppose that $T \sim R^{-\beta}$ in the distant future. From equation (4), absorption is complete if $[4+(\alpha+1)\beta]n \leq 1$. Davies^{2,6} investigated the thermal future of the universe by considering the interaction of the intergalactic medium with the cosmic electromagnetic blackbody background radiation. He showed that for models that expand more slowly than $t^{1/2}$, the intergalactic plasma eventually completely recombines; for models that expand more rapidly than $t^{1/2}$, the plasma does not completely recombine and remains coupled to the electromagnetic background radiation. If the intergalactic medium eventually recombines, then $\alpha = 1/2$ and $\beta = 2$; if the medium does not recombine, then $\alpha = -3/2$ and $1 \leq \beta \leq 2$. Thus, absorption of retarded gravitational waves in Class II universes is complete if $n \leq 1/7$.

Now consider advanced gravitational waves. Assume that σ_e approaches a constant as $\omega \rightarrow \infty$. In Class I universes, expression (5) shows that absorption is finite for all values of n . In Class II universes with a "hot big-bang", as they travel into the past, advanced waves that are not absorbed first eventually enter an epoch in which the cosmic matter is in thermal equilibrium with electromagnetic radiation at relativistic temperatures. As this epoch is entered there is a rapid increase in N because of thermal electron-positron pair production⁷; within this epoch the mass density of the cosmic medium varies as R^{-4} , but N still varies as R^{-3} . Thus full absorption of advanced waves occurs for $n \geq 1/3$.

Consider now condensed objects, and suppose that their interaction with gravitational waves can be represented by a constant σ_e . Suppose that in Class I universes there is a constant number density of such objects: for retarded waves absorption is complete; for advanced waves, absorption is finite. Suppose that in Class II universes the number density of condensed objects varies as R^{-3} in the distant future: absorption of retarded waves is complete for $n \leq 1/3$.

Assume, for the sake of argument, that the absorber theory of radiation is valid for gravitational waves, that only retarded gravitational waves can be observed in the actual universe and that σ_e saturates at high frequencies. In determining what cosmological models are to be eliminated, with these assumptions, as possible models of the Universe, the relevant values of n are those applicable for $t \rightarrow 0$ and for $t \rightarrow \infty$; such values will be denoted by n_0 and n_∞ respectively. No Class I universe with the characteristics taken above is eliminated. Class II universes with $n_0 > 1/3$ are eliminated. Class II universes with $n_\infty > 1/3$ are eliminated. In the standard "hot big-bang" model of general relativity, $n = 1/2$ in the radiation-dominated epoch so that this model has consistent advanced fields and is eliminated. In the spatially flat case of the Brans-Dicke cosmology, $n = (2\omega_{BD} + 2)/(3\omega_{BD} + 4)$ where ω_{BD} is a parameter; with⁸ $\omega_{BD} \geq 0$ it follows that $1/2 \leq n \leq 2/3$, where the upper limit corresponds to the Einstein-de Sitter universe of general relativity: these models are eliminated since retarded fields are inconsistent. The Friedmann models with zero cosmological constant and negative spatial curvature have $n_\infty = 1$ and are hence eliminated.

Of course, these eliminations are purely hypothetical, being based on the three assumptions mentioned above. If cosmological observations were to establish one of these hypothetically eliminated models as a reasonable model of the Universe, then that result would constitute evidence against at least one of the assumptions.

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Solar test of Dirac's large numbers hypothesis

DIRAC^{1,2} has proposed that the large dimensionless numbers that are constructable from the fundamental constants of physics and astronomy are related to each other, and are simple functions of our present epoch in the Universe. Thus he suggests that, as measured by atomic standards, the gravitational constant, G , varies with the time as t^{-1} and the number of nucleons in the Universe increases as t^2 . In more recent work^{3,4}, he has pursued the consequences of assuming different modes of creation of new matter. We have found that Dirac's theory of multiplicative creation, but not his theory of additive creation, is not in contradiction with known facts about the Sun.

By "additive creation" he implies that matter is formed uniformly throughout space, and therefore mostly in intergalactic regions; the mass of a small object like a star would remain essentially unaltered by the new creation of matter. "Multiplicative creation" means that existing matter multiplies itself in proportion to the amount of matter already present; therefore the mass of a star, M , would grow as t^2 .

In view of the important cosmological consequences of Dirac's theory, it is desirable to test it with methods presently available. One useful test object is the Sun. The proposed large change in G over the Sun's past lifetime and, with multiplicative creation, the concomitant large change in the Sun's mass, would be expected to produce a different evolutionary history for the Sun as compared with the 'standard' history computed on the basis of constant G and M . But with any theory the observable properties of the Sun today (mass, luminosity, and radius) must be achievable with 'reasonable' choices of initial chemical composition. Unfortunately, the chemical composition of the present surface layers, which presumably reflect the original composition, is known only with great uncertainty, and this allows a considerable latitude of choices. A further constraint (and a possible benefit that could accrue from a 'non-standard' theory) is the small solar neutrino flux that is observed and is not explained by 'standard' theories.

The one 'free' parameter in Dirac's theory is the age of the Universe, t_0 , although observations of the Hubble constant put some limits on it. If t is measured from the time of formation of the Sun, then

$$G(t) = G_0 \left[\frac{t_0 - t_\odot + t}{t_0} \right]^{-1}$$

and

$$M(t) = M_0 \left[\frac{t_0 - t_\odot + t}{t_0} \right]^n$$

with $n=0$ for additive creation and $n=2$ for multiplicative creation. At the present epoch, $G_0 = 6.67 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1} \text{ s}^{-2}$, $M_0 = 1.99 \times 10^{33} \text{ g}$, and $t_\odot = 4.5 \times 10^9 \text{ yr}$.

The hypothesis of additive creation has already been tested by Pochoda and Schwarzschild⁵. They found that this hypothesis can be satisfied if $t_0 \geq 15 \times 10^9 \text{ yr}$. Today, such an age for the Universe is certainly admissible, although it seemed rather too long a decade ago. But the required initial hydrogen content, $X = 0.81$, seems somewhat high. Moreover, the predicted brightness of the Sun in the past, in spite of the greater distance

of the Earth from the Sun then (in the additive theory), may conflict with palaeontological evidence⁶. One further consequence of the additive theory is particularly relevant here: the hydrogen content near the centre of the present Sun must be completely exhausted. Although Pochoda and Schwarzschild did not publish any temperatures, the central temperature of their final model is probably about 19×10^6 K (see, for example, ref. 7). In these conditions the expected neutrino flux must be enormous—several orders of magnitude greater than the upper limit of the neutrino flux measured by Davis⁸. But it should be borne in mind that standard solar models themselves predict a neutrino flux that is about an order of magnitude larger than Davis's upper limit of 1 SNU.

In order to test Dirac's hypothesis of multiplicative creation, we have evolved several sequences of stellar models up to the present age and mass of the Sun. It has been assumed that, since the solar gases are highly ionised, new ions (and a corresponding number of electrons) are created in the vast empty spaces between the existing particles. They are assumed to be of the same species as their 'parent' ions. Moreover, they must share the same kinetic energy and linear momentum as their neighbours, on the average, and therefore units of kinetic energy and momentum (including units of rotational angular momentum of the whole Sun) must also be created. Dirac has already postulated these conditions for the Earth, in order not to contradict geological evidence. For our purposes, it is sufficient merely to scale up the chemical structure of the evolving solar models as the mass of the Sun is increased. For simplicity, axial rotation of the Sun is neglected.

The other physical input parameters are the same as those used in previous work⁹. To summarise them here for convenience, we have adopted: the new Carson (unpublished) opacities, which are very close to those of Cox and Stewart¹⁰ for main-sequence stars of low and intermediate masses; the nuclear cross sections of Bahcall, Bahcall and Ulrich¹¹, but with the neglect of the *pep* reaction; a ratio of mixing length to pressure scale height in the outer convective envelope equal to $\alpha = 2$; and a metals abundance of $Z = 0.015$. Results for the initial and final solar models are given in table 1. We have found that the final radius does not agree exactly with the observed radius, for the choice of $\alpha = 2$. But, as many people have shown a suitable change of α can achieve agreement between the radii without significantly altering the luminosity, which is affected primarily by the choice of initial chemical composition. Therefore, we have not considered it worthwhile to rerun the sequences with different guesses for α until the final radii are correct.

The standard solar model obtained here is very similar to the analogous one already calculated by Carson, Ezer, and Stothers⁹. The two models are not identical because we have here adopted equilibrium nuclear reaction rates and a slightly different treatment of the thermodynamical quantities, apart from the fact that the computer program itself is different.

Turning now to the final solar models based on Dirac's multiplicative theory, we find the rather surprising result that they are nearly the same as the final model based on standard

theory! This occurs in spite of the widely disparate initial masses, occasioned by the use of a full range of choices for t_0 . The reason for this similarity is that the effect of a larger G in the past is to increase the luminosity⁶, whereas a lower stellar mass decreases it. As a result of these compensating factors, the ratio L/M remains approximately constant. Since the nuclear reaction rate and the amount of hydrogen depletion depend on the L/M ratio¹², the final central temperature and the final hydrogen profile throughout the Sun are about the same as they were in the standard case.

Since the radius, r , of the Earth's orbit around the Sun increases, in Dirac's multiplicative theory, as t , the temperature of the Earth's surface may be expected to change like $(L/r^2)^{1/4} \sim \text{constant}$. This naive prediction is not in contradiction with palaeontological evidence. But why are well-preserved Precambrian and early Cambrian fossils in essentially perfect shape if their masses have increased by a significant percentage?

One could pursue the test of Dirac's theory by constructing theoretical isochrones for old clusters containing stars of low mass and comparing them with observed cluster H-R diagrams. Although we have not done this, it is interesting that the more massive members of this old population must have evolved long ago—mostly into white dwarfs, initially. But since the upper mass limit for a stable white dwarf (or neutron star) is proportional to $G^{-3/2} \sim t^{3/2}$, and since the mass of the star itself increases as t^2 , eventually these stars will all undergo gravitational collapse. The galactic halo must be popping with continually forming black holes. The ultimate cosmological consequence is a universe full of gravitationally collapsed objects.

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Table 1 Final models for the present Sun based on Dirac's theory of multiplicative creation

Case	Standard	1	2	3
t_0 (10^9 yr)	—	20	10	7
Initial G/G_0	1	1.29	1.82	2.80
Initial M/M_0	1	0.60	0.30	0.13
X	0.758	0.755	0.751	0.749
Z	0.015	0.015	0.015	0.015
Initial $\log L/L_\odot$	-0.13	-0.43	-0.83	-1.35
Initial $\log T_c$	3.73	3.71	3.67	3.62
Final X_c	0.40	0.43	0.45	0.47
Final T_c (10^6 K)	15.2	15.1	15.1	15.0
Final ρ_c (g cm^{-3})	151	144	140	133
Neutrino flux (s.n.u.)	~ 7	~ 6	~ 6	~ 5

Age and rates of denudation of Trap Series basalts at Blue Nile Gorge, Ethiopia

BETWEEN Lake Tana and the Sudan border the Blue Nile has cut a deep gorge across the Ethiopian Highlands. The first 150 km of its course lies on lavas of the Tertiary Trap Series, after which the river flows through horizontal Mesozoic and Palaeozoic sediments until the Precambrian is reached 330 km from Lake Tana¹. About 280 km below the lake the Addis Ababa-Debre Markos road crosses the gorge at $10^\circ 05'N$, $38^\circ 10'E$, where 250 m of Trap Series basalts overlie at least 1,150 m of sediments the base of which is not exposed. The gorge is 1,400 m deep and 20 km wide and is incised into a plateau 2,600 m high.

On the south side of the gorge the Trap Series section comprises at least five thick horizontal flows of aphanitic basalt, belonging to the lower part of the Trap Series as a whole. The top three flows are well exposed and each is several tens of metres thick and extends laterally for over

Table 1 Potassium-argon ages on whole rock samples of basalt from the left bank of the Blue Nile Gorge near the crossing of the Addis Ababa-Debre Markos road, Ethiopia

Field location	Laboratory no.	Weight %	Rad. ^{40}Ar (10^{-7} cm 3 NTP g $^{-1}$)	100 Rad. ^{40}Ar Total ^{40}Ar	Calculated age (Myr) $\pm$ 2 s.d.	Approximate percentage glass or mesostasis
Top flow	72-555	0.739, 0.743	6.99	62.7	23.5 $\pm$ 0.3	30
Second flow	73-667	0.919, 0.925	8.97	82.1	24.2 $\pm$ 0.4	15
Third flow	73-668	0.776, 0.772	7.15	78.1	23.0 $\pm$ 0.3	20
Fourth flow	73-669	0.888, 0.891	9.73	84.6	27.2 $\pm$ 0.4	10
			9.80	84.0	27.4 $\pm$ 0.4	

$$\lambda_e = 0.585 \times 10^{-10} \text{ yr}^{-1}; \lambda_\beta = 4.72 \times 10^{-10} \text{ yr}^{-1}; {}^{40}\text{K}/\text{K} = 1.19 \times 10^{-2} \text{ atom } \%$$

5 km. These features suggest that the flows were the product of fissure eruptions².

Grasty *et al.*² tried to date the flows, but their results were inconclusive. We have dated fresh samples from the top four flows (Table 1), using techniques described previously³. It was not possible to obtain samples free from interstitial glass or mesostasis, and the possibility of argon losses must be taken into account when interpreting the results, notably the low age obtained for the third flow. Nevertheless, the close agreement in ages obtained suggests strongly that they are near the truth, and that the actual age of the basalts is certainly less than 30 Myr. This age is similar to the ages obtained⁴ from the lower part of the Trap Series elsewhere in Ethiopia.

The dating of these horizontal flows gives a lower limit to the age of incision by the Blue Nile into the uplifted Ethiopian highlands. Merla⁵ considered that uplift occurred in two stages: one in the Miocene, and the other in the Plio-Pleistocene with the development of deep canyons, including the Blue Nile and Tekezze (Atbara) gorges. The overall rate of erosion since the deposition of these basalts may be calculated by two methods. One is to find the volume of sediments derived from the upland Blue Nile and Tekezze basins and deposited in the Gezira and Atbara fans, along the main Nile and in the Nile Delta. The other is to calculate the volume of rock eroded from the uplands themselves.

At present the White Nile provides less than 2% of the annual sediment load of the main Nile⁶, and the high proportion of Ethiopian minerals⁷ and pollen⁸ in Nile deltaic sediments indicates that a similar situation obtained during at least part of the Cainozoic. Various estimates of the volume of sediments comprising the Nile Delta have been made^{9,10}, but taking into account seismic work¹¹, a figure of between 100,000 and 200,000 km³ seems reasonable. The volume of alluvium along the main Nile floodplains and terraces between Khartoum and the delta is not known, but an estimate of between 100 and 600 km³ can be made. The Gezira Plain between the Blue Nile and the White Nile south of Khartoum is a low-angle alluvial fan built up mainly by the Blue Nile¹². Isopach values¹³ indicate a volume of about 1,800 km³, which excludes probable White Nile alluvium. A rough estimate of 800 km³ may be made for the volume of the Atbara Fan, given its smaller catchment area relative to the Blue Nile.

The total volume of material deposited by the Blue Nile and Atbara (Tekezze) in Egypt and Sudan is, therefore, estimated at between 98,000 and 225,000 km³, the bulk of which forms the Nile Delta. For simplicity we here assume a value of 150,000 $\pm$ 50,000 km³, derived mainly from Ethiopia. The areas of the two basins in upland Ethiopia above 1,000 m are 190,000 and 85,000 km², respectively. Allowing for the difference in bulk density between Nile deltaic sediments (about 1.5 g cm⁻³) and the eroded Ethiopian bedrock (about 2.8 g cm⁻³), and assuming a 30% loss in solution from the Ethiopian uplands, the mean erosion rate over the past 23.5 Myr would be $12 \pm 6 \text{ m}^3 \text{ km}^{-2} \text{ yr}^{-1}$.

This result may be compared with that obtained by calculating the volume of rock eroded from the two basins, using a contour map of the highlands. The dissected plateau has a mean local relief of about 1,500 m and a mean surface elevation of about 2,500 m. The estimated volumes of rock removed from the Blue Nile and Tekezze basins are 71,000 km³ and 31,000 km³, respectively. That gives a total of 102,000 km³, to an accuracy of about 50%. As the area of the basins is about 275,000 km², the mean rate of denudation must have been $15 \pm 7.5 \text{ m}^3 \text{ km}^{-2} \text{ yr}^{-1}$.

Such denudation rates of around 0.015 mm yr⁻¹ are very low for a tectonically active region of high relief¹⁴⁻¹⁸; they are more in keeping with modern rates from undisturbed, forested, tropical lowlands¹⁹⁻²¹. Modern erosion rates in the region are far higher, with estimates of the Nile sediment load at Cairo ranging from 57 to 120 Mt yr⁻¹ (refs 6, 22 and 23). These estimates show a doubling of sediment load during the past 70 yr^{23,24}, reflecting accelerated erosion of the headwaters. If an annual sediment load of 50-100 Mt is derived from Ethiopia, the present rate of erosion in the headwaters of the Blue Nile and Tekezze must be about 120-240 m³ km⁻² yr⁻¹, or an order of magnitude greater than the overall geological rate.

The discrepancy between the two rates suggests episodic uplift interspersed between prolonged stable intervals. Faure²⁵ calculated the rate of uplift of the plateau to be 0.5-1.0 mm yr⁻¹ during the Upper Pleistocene, compared with a mean rate of 0.1 mm yr⁻¹ since the Middle Tertiary. We conclude that a change in the rate of uplift of that magnitude, coupled with recent deforestation of the headwaters, can explain adequately the difference between the modern and geological erosion rates. It may be further noted that the close agreement between the volume of material eroded from Ethiopia, and the volume in the Nile Delta, is further evidence suggesting that the bulk of the Nile deltaic sediments are of Ethiopian provenance.

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Non-etching optical detection of fission tracks using Teflon

THE detection of tracks in material irradiated by charged particles, has been accomplished using electron microscopy¹ and etching followed by optical microscopy²⁻⁶. We have attempted to detect unetched tracks using optical microscopy. Presumably, unetched tracks retain more information about the radiation than is retained after etching.

The kinds of damage which can be caused by the passage of a charged particle through any material are bond ruptured, which produces free radicals⁷, physical dislocations, as in the Wigner effect in reactors in which carbon atoms in graphite are displaced by fast neutrons, and certain other less obvious but more visible effects such as the production of metastable states which emit light, as in thermal luminescence and scintillation counters.

It seemed possible that, in order to obtain the best record of the track itself, a material and detection process could be used which would minimise the general background and make possible the direct observation of the track itself using an optical microscope. So we chose, following the work of others⁸⁻¹², to use cross linkage copolymerisation of radiation-damaged polytetrafluoroethylene, Teflon, with acrylic acid monomer followed by dyeing with the basic dye Rhodamine B which phosphoresces in orange under exposure to blue light. Teflon is one of the most chemically resistive materials known and should give essentially no background.

Teflon plastics films (51, 127, and 762 μm thick) were irradiated in air with fission products from a 50 μCi ²⁵²Cf source for 1–2 min at room temperature. The samples were then inserted into glass tubes containing acrylic acid solution and degassed by freezing and pumping the system

several times. After degassing, a grafting reaction with the free radicals in the tracks was allowed to continue in the absence of air at controlled temperatures (23–40 °C), for varying times (5–30 h). Good grafting was obtained at 23 °C after 25 h or at 40 °C after 5 h. We were also successful in getting good grafting results when the reactions were carried out in a dry nitrogen atmosphere. In these experiments dry nitrogen gas was bubbled through the acrylic acid solution for 15 min and then the tube was closed. Grafting occurred in dry nitrogen under conditions similar to those used in the evacuation experiments.

The solution was 50% vacuum distilled acrylic acid ($\text{CH}_2\text{CH}_2\text{COOH}$). After the grafting, the Teflon samples were washed thoroughly in deionised water at 50 °C for 2 h. Following this they were dyed with a boiling 3% solution of Rhodamine B for several hours and then washed for 15 min in a 2% acid soap solution to remove any unfixed dye. This procedure was apparently successful: the basic dye fixed itself to the acidic polymer that was attached to the damaged area of the Teflon.

Finally, the samples were examined with a Reichert-Austria Zetopan microscope fitted with an ultraviolet lamp. Rhodamine B phosphoresces with an orange colour when illuminated with light at a wavelength of 400 nm. A cutoff filter eliminated the excess blue light which produced a dark field, and the fission tracks then appeared as bright lines (Fig. 1a and b).

It seems possible that more information can be obtained from tracks developed and measured in this way and that Teflon treated like this may well be useful in the future detection of tracks formed by the passage of charged particles.

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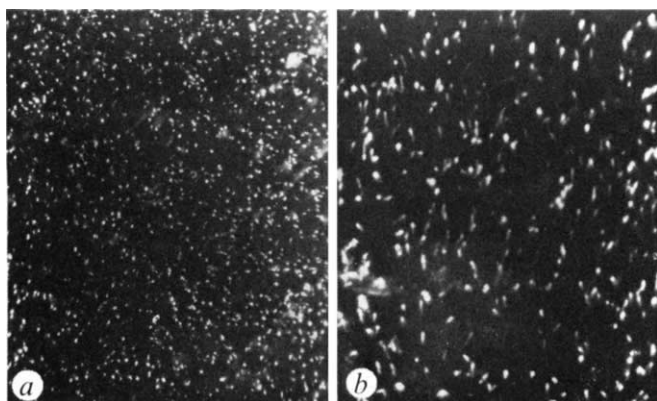
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Fig. 1 Tracks of fission fragments. a, $\times 180$ diameters; b, $\times 360$ diameters. Made by grafting acrylic acid on to Teflon. The apparent length of each track is related to the angle of its inclination.



Rapid hydrogenation of sterols in a contemporary lacustrine sediment

THE steroid carbon skeleton has been widely used as an indicator of biological origin of the organic material in ancient sediments¹⁻³ and petroleum⁴. Sterols, presumed precursors of the geolipid steranes, and stanols, their saturated analogues, have been identified in Recent⁵⁻⁷ and ancient^{8,9} sediments. In view of their generally low abundance in living

organisms, the presence of stanols in Recent sediments suggests the operation of a sterol hydrogenation process early in the geological time scale. We have sought to confirm this hypothesis by examination of the contemporary sediments of Rostherne Mere, Cheshire, England. The sterol composition of the sediment, and its variation with depth, provide circumstantial evidence for the hydrogenation of Δ^5 -sterols¹⁰. Here we describe the application of radiochemical techniques^{11,12} to the study of the early diagenesis of cholesterol (cholest-5-en-3 β -ol) in Rostherne sediment and report the first direct evidence of rapid sterol hydrogenation in a geological environment. The sediment chosen for study is that of a highly productive lake. Significant allochthonous (land-derived) organic input to Rostherne Mere has been exceeded in recent years by an increasing autochthonous production of blue-green algae^{13,14}. The sediments in the deepest part of the lake (from where samples were taken) remain severely depleted in oxygen throughout the year¹⁵. As a National Nature Reserve, Rostherne Mere is protected from gross pollution, though there is some chemical evidence to suggest a recent input of petroleum-derived material¹⁴.

4-¹⁴C-cholesterol (Radiochemical Centre, Amersham) was purified by thin-layer chromatography on silica gel and on silver nitrate impregnated (30% by weight) alumina (Ag⁺TLC)¹⁶, to remove traces of ¹⁴C-stanols. Incubations with 4-¹⁴C-cholesterol were conducted both in sediment cores from Rostherne Mere and in the model environment of anaerobic sewage sludge. Sediment cores (50 cm $\times$ 6 cm), encased in Perspex tubes, were taken intact from the deepest part of Rostherne Mere. For laboratory incubation, the core was stored in a polystyrene container, over a small quantity of dry ice, during transport to the laboratory. A second sediment incubation involved injection of 4-¹⁴C-cholesterol and suspension of the core in the lake near the sediment-water interface for the duration of the incubation. The "zero time" incubation, in which acidification and extraction (heptane-isopropanol) immediately followed injection of radiolabel, enabled an assessment of purification and work-up procedures. Further experimental details are given in Tables 1 and 2; a full discussion will be provided elsewhere (S. J. G. and G. E., in preparation).

Table 1 summarises the sterol constituents identified in Rostherne sediment. Examination of the variation in sterol composition indicates an increase in the stanol to Δ^5 -sterol ratio with sediment depth (ref. 10 and S. J. G. and G. E., in preparation). For the 0–10 cm sediment level, examined in conjunction with a labelling experiment (A, Table 2), the carbon number distributions of the Δ^5 -sterol, 5 α -stanol and

Table 1 Sterol constituents of Rostherne sediment*

Structural type†	Abundance (p.p.m.) of constituent‡		
	C27	C28 (24-methyl)	C29 (24-ethyl)
Δ^5 -sterol	20	11	24
$\Delta^{5,22}$ -sterol	1	7	5
Δ^{22} -sterol (5 α)	?	10	8
5 α -stanol§	47	22	49
5 β -stanol	9	3¶	9

* Constituents of a 0–10 cm sediment core (experiment A, Table 2). Individual sterols identified on the basis of gas chromatography (GC) retention times (trimethylsilyl ethers) and mass spectra (obtained by gas chromatography-mass spectrometry (GCMS) of the trimethylsilyl ethers facilitated by computer accumulation and interpretation of data^{14,17,18}). Authentic standards available for those components for which amounts are given in italics.

† Δ^5 -sterols (with $\Delta^{5,22}$ -sterols) separated from 5 α -stanols (with Δ^{22} -sterols) and 5 β -stanols by Ag⁺TLC. Fraction most strongly retained on Ag⁺TLC not examined.

‡ Constituents designated by carbon number. "?" indicates lack of positive identification. Abundances expressed as p.p.m. dry weight of extracted sediment.

§ C₂₈ stanol observed at lower sediment depth (ref. 10, and S.J.G. and G.E., in preparation).

¶ Tentative assignment.

Table 2 Incubation of 4-¹⁴C-cholesterol in Rostherne sediment and anaerobic sewage sludge

Radiolabel	Experiment*			
	A (sediment)	B (sediment)	C (sewage)	D (sewage)
Total activity injected (μ Ci)†	14.67	3.10	3.61	3.23
Incubation products:‡				
total lipids (%)	69	29	77	75
¹⁴ C-cholesterol (%)	45	nd §	28	68
¹⁴ C-5 α -cholestanol (%)	0.47	0.09	0.66	—¶
¹⁴ C-5 β -cholestanol (%)	0.11	0.03	1.2	0.06

* A, Incubation (90 d, 10 °C) in intact core in the laboratory (dark), injection at 4 cm sediment depth; B, incubation (65 d, 5.4 °C) at lake bottom, injection at 4 cm sediment depth; C, incubation (28 d, 37 °C) in the laboratory (dark); D, "zero-time" incubation (see text).

† Injections of 4-¹⁴C-cholesterol made in Tween 80 suspension.

‡ Expressed as percentage of radiolabel injected; no allowance made for experimental losses. Total extracts separated by shaking with aqueous KOH (7%) and neutrals further separated by silica TLC. " Δ^5 -sterols", "5 α -stanols" and "5 β -stanols" obtained from crude total sterol fraction by Ag⁺TLC. Radiolabelled stanol products of incubation characterised by radio-TLC, radio-GLC and GC-MS (S.J.G. and G.E., in preparation). Other radiolabelled products uncharacterised or not fully characterised; see text.

§ Not determined.

¶ Radioactivity not detected above background.

5 β -stanol constituents are similar, confirming the earlier findings¹⁰. The presence of large quantities of stanols, relative to unsaturated sterols, together with the parallel distributions of saturated and unsaturated analogues provides strong evidence for the operation of an hydrogenation process in the young Rostherne sediment.

The results of radiolabelling experiments are summarised in Table 2. The incomplete recovery of radiolabel in the zero time incubation experiment D emphasises the difficulty of quantitative extraction of sterols. The particularly low recovery in experiment B, where incubation was conducted in the lake, may be attributable to losses at the flocculent sediment-water interface. In sediment incubations, unchanged ¹⁴C-cholesterol was the major labelled product but sewage incubation (experiment C) yielded a major fraction (31% of injected radioactivity) corresponding in polarity to unsaturated steroid ketones (see below). Each stanol fraction, obtained by Ag⁺TLC, was subjected to a second Ag⁺TLC separation to ensure absence of ¹⁴C-cholesterol. The "5 α -stanol" TLC fraction from zero-time incubation (D, Table 2) contained no detectable activity. The origin of the small quantity of activity in the "5 β -stanol" fraction is uncertain but may arise from TLC overlap with products of sterol dehydration during the work-up procedure. The levels of activity in the stanol fractions from sediment and sewage incubations (A and C, Table 2), however, indicate clearly that both 5 α - and 5 β -stanols are genuine products of incubation. In experiments A and C, analyses by radio-gas chromatography and combined gas chromatography-mass spectrometry (GC-MS) established ¹⁴C-5 α -cholestanol and ¹⁴C-5 β -cholestanol as the sole radiolabelled components of their respective fractions. The 5 α -isomer predominated, both as a radiolabelled product of incubation and as a natural constituent, in the sediment incubation whereas 5 β -cholestanol was the more abundant in sewage sludge.

In both sediment and sewage incubations, other labelled products of incubation included components whose TLC mobilities were consistent with saturated and unsaturated steroid ketone structures. Radio-gas liquid chromatography (GLC) analyses with coinjection with authentic standards suggested the presence of ¹⁴C-cholest-4-en-3-one, ¹⁴C-5 α - and ¹⁴C-5 β -cholestanone. This evidence suggests that the hydrogenation of the Δ^5 double bond in cholesterol, effective in sediment and sewage sludge, may be a biochemical process for which the mechanism is similar to that observed to be

operative in certain bacterial^{19,20} and other biological systems^{21,22}.

We believe that the results of the radiolabelling experiments with ¹⁴C-cholesterol in Rostherne sediment provide a clear indication of an hydrogenation process taking place in the sediment under environmental conditions, for the following reasons. First, the radiolabelling results are consistent with the indirect evidence for a sterol hydrogenation process based on sediment lipid analyses. The relative amounts of Δ^5 -sterols and 5 α -stanols (in approximate ratio 1:2), as natural sediment constituents, suggest a conversion of approximately 60% during the period of deposition of the 0–10 cm sediment layer (about 20 yr), indicating an average rate of hydrogenation approximately equal to that observed in the labelling experiment (A) (that is, 0.5% in 3 months).

Second, the specific activities (determined by radiocounting and GLC quantitation, with an internal standard) of radiolabelled 5 α -cholesterol and 5 β -cholesterol, following incubation of ¹⁴C-cholesterol in Rostherne sediment (experiment A) were the same (0.11 μ Ci mg⁻¹).

Third, incubation of ¹⁴C-cholesterol in a sediment core, suspended in the lake for the duration of the incubation (experiment B), also indicates conversion to radiolabelled stanols.

These experiments have demonstrated that the geochemically significant hydrogenation of naturally-occurring unsaturated sterols, to give both 5 α - and 5 β -stanols, may be effected extremely rapidly in contemporary aquatic sediments. The presumably microbiological process may be analogous to that observed in other biological systems with the consequent 5 α :5 β -stanol ratio dependent on the particular microbial population (compare the results of ¹⁴C-cholesterol incubation in anaerobic sewage sludge).

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The hipparions of the Baringo Basin sequence

THE first appearance of *Hipparion* in land mammal populations of the northern hemisphere is one of the best documented biochronological events of the Neogene: the genus appeared first in the Mediterranean world about 12.5 Myr ago¹. In France² its first appearance was during the Middle–Upper ‘Helvetian’ (ante-‘Tortonian’). New radiometric information from the Far East supports an age of 10.5 Myr for the base stratotype Tortonian³. This means that the *Hipparion* datum of 12.5 Myr is some 2 Myr earlier than the advent of the Vallesian.

In North Africa the earliest *Hipparion* is the same as the primordial Old World *Hipparion primigenium* (Von Meyer) of the ‘Pontian’ (Vallesian and Pikermian) of Europe. It was described as *Hipparion africanum* Arambourg³ from the Vallesian of Wad el Hammam in Algeria⁴. *H. primigenium* also occurs in the Tortonian Beglia Formation of Tunisia⁵.

In Asia, *Hipparion* appeared first in the Nagri Formation of the Siwaliks⁶ about 10–12 Myr ago. Hussain described the Nagri Formation *Hipparion* as *Hipparion nagriensis*⁷. Although the skull is unknown, *H. nagriensis* seems to be a clear synonym of *Hipparion primigenium*: the cheek teeth are of the same size and have the very characteristic pattern of this species (see ref. 7 plates 1 and 2, and ref. 8 Figs 21 and 22). Hussain noted the resemblance of *H. nagriensis* to *Hipparion catalaunicum* Pirlot and *H. koenigswaldi* Sondaar⁷, both of which are synonyms of *H. primigenium*⁴.

In the summer of 1973 I received the *Hipparion* remains from the Ngorora Formation in Kenya, which is between 12 and 9 Myr old⁹ (see also ref. 10). Before this discovery, it was thought that the earliest *Hipparion* south of the Sahara was in the Mpesida Beds of Kenya, dated at 7 Myr, terminal Pikermian^{9,11}. The *Hipparion* from the Mpesida Beds is the same as that from Lothagam-1 in the Turkana District on the west side of Lake Rudolf in Kenya, age 6 Myr. It has been described as *Hipparion turkanense*^{12,13}.

The Ngorora *Hipparion* is very different from *H. turkanense* but is indistinguishable from *Hipparion primigenium*. *Hipparion primigenium* also occurs in the Chemeron Formation (locality J.M.493), at Kanapoi, and in the Aterir Beds. On the other hand, teeth indistinguishable from *H. turkanense* are found in the Kaperyon Beds. Thus, it seems that there are two large species of *Hipparion* in the Baringo Basin sequence: *H. primigenium* emerging in the Ngorora Formation, and *H. turkanense* in the Mpesida Beds, representing a later invasion from Asia. The two species survived till the 4 Myr level, for teeth indistinguishable from those of *H. turkanense* are found at Kanapoi and in the Mursi Formation of southern Ethiopia. With the discovery of *Hipparion primigenium* in the Ngorora Formation it has been settled that there is not so much of a time lag between the first appearance of *Hipparion* south of the Sahara and that north of the Sahara as had been supposed previously. One species only, *Hipparion primigenium* (Von Meyer), first appears to be distributed over much of the Old World, not only in Eurasia but also in Africa both north and south of the Sahara in the Vallesian.

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Distribution of hydrogen bond angles in molecular crystals

THE structures of hydrogen-bonded molecular crystals have been studied extensively, and the resulting histograms of the distributions of the O–H···O bond angles θ show maxima at approximately 15° from the linear configuration^{1–3}. At first sight this seems odd, because theoretically a linear hydrogen bond is stable^{4,5}. Kroon and Kanters³ have indicated, however, that because the number of possible hydrogen bond configurations at any value of θ is proportional to $\sin\theta$, these histograms should not be interpreted to indicate that the configuration of $\theta=15^\circ$ is, energetically, the most stable. They applied the $\sin\theta$ correction to the statistics of a series of 196 values of hydrogen bond angles in molecular crystals, and showed that the experimental distributions are not inconsistent with an assumed preference for linear hydrogen bonds.

One of us (M.H.)⁶ has calculated the potential energy function for the bending of the hydrogen bond O–H–O···O–H₂ using the method of CNDO/2, one of the semi-empirical LCAO MO SCF methods which includes all the valence electrons. We intend to show here that the histograms of Kroon and Kanters³ can be explained by Hasegawa's⁵ potential energy function.

The potential function⁵ can be represented approximately by the following quadratic form:

$$\Delta E = s\theta^2/2 \quad (1)$$

in which the linear hydrogen bond is most stable and ΔE represents the strain energy for the bending through θ degrees of the hydrogen bond. The value of the force constant, s , is estimated at 0.4 eV/rad^2 . In the case of the hydrogen-bonded water dimer, the dependence on the angle ω of the strain energy ΔE is small, so that factor is neglected here.

If it is assumed that the distribution among configurations in various molecular crystals may be approximated by a Boltzmann distribution, the distribution function $f(\theta)$ for the bending of the hydrogen bonds is represented by

$$f(\theta) = C \sin\theta \exp(-s\theta^2/2kT) \quad (2)$$

where C is the normalisation factor, k is the Boltzmann constant, and T is temperature, taken to be 300 K. The factor, $\sin\theta$, is necessary because the number of possible configurations at a given value of θ is proportional to $\sin\theta$ and the energy dependence on ω is neglected.

The histogram for the distribution of the O–H···O hydrogen bond angles can be calculated using this procedure (Fig. 2).

Figure 2 also shows Kroon and Kanters's histogram³ of 196 O–H···O hydrogen bond angles observed in molecular

Fig. 1 A bent hydrogen-bonded configuration.

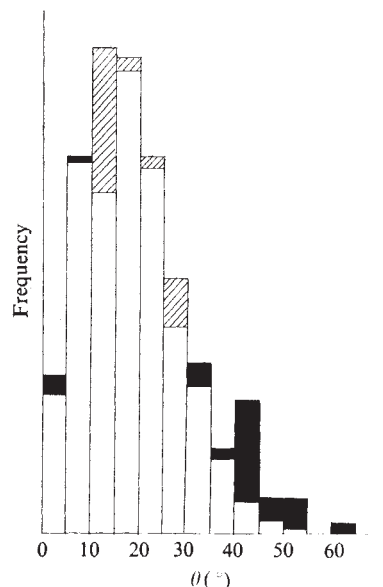
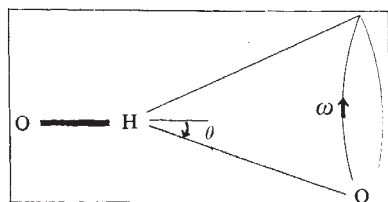


Fig. 2 The superimposed histograms of the theoretical and experimental distributions of O–H···O hydrogen bond angles. The experimental histogram is from Fig. 2 of ref. 3. Excess of experimental frequency over theoretical frequency is indicated by solid shading and excess of theoretical frequency over experimental frequency is indicated by hatching. Thus, open column indicates the amount of frequency common to both theory and experiment.

crystals. The two histograms agree quite well with each other: in our calculation the distribution shows a maximum at 14.5° , and the maximum from the molecular crystal data is approximately 15° . The slightly narrower distribution of our theoretical histogram compared with the experimental histogram may be because of the omission of the higher order terms in the right hand side of equation (1).

The hydrogen bond angle is a factor of the molecular structure and molecular arrangement in each crystal. The fact that the distribution of θ , found when the values of the hydrogen bond angle in various crystals are treated statistically, coincides with the distribution for an isolated hydrogen bonded structure, seems to suggest that the perturbation experienced by a structure of O–H···O in molecular crystals is very small.

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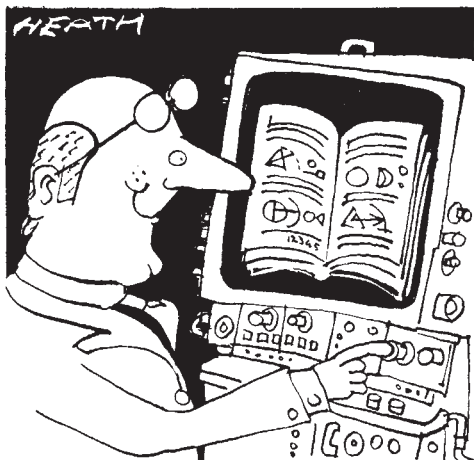
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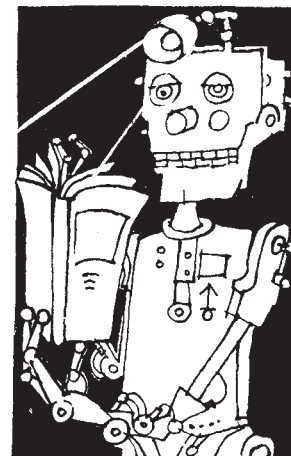
Marks of unknown carbonate-decomposing organelles in cyanophyte borings

BLUE-GREEN algae are a major agent of destruction of carbonate rocks and sediments, particularly in the littoral and shallow marine environments. The organisms discussed here are endolithic, that is, the individual algal filaments reside snugly in made-to-measure tunnels of their own, excavated chemically. Data presented here suggest that much of this geologically significant bioerosion is actually caused by what may be called digestion of the mineral substance, and is not,

(Continued on page 237)



Book review supplement



IN spite of recent advances in the study of the history of education, many important tendencies and crucial decisions have never been fully explored. There is also a dearth of good biographies. The intelligent and industrious partnership of Ashby and Anderson has now provided, however, a lively and valuable contribution to this field. Their book is scholarly and compact and is based on a careful examination of a mass of scattered evidence.

Haldane was a front rank Liberal politician, more remembered for his army reforms than for his educational activities. Even his own autobiography has relatively little to say about education. Yet he was concerned throughout his life more with education than with any other topic and with the ends of education as much as with the means. He placed it in the forefront both of industrial progress and of democratic politics, and with a rare combination of psychological and political skills initiated or participated in many practical educational enquiries and enterprises. The very range of his activities and the extent of his commitments make his occasional failures as interesting and significant as his many successes, and the decisions which were not taken as a result of his advice or actions are as worthy of thoughtful investigation as the decisions which were.

Haldane believed profoundly in the unity and continuity of education—including adult education about which he had much to say. He also believed in a bigger place for science and technology within university institutions.

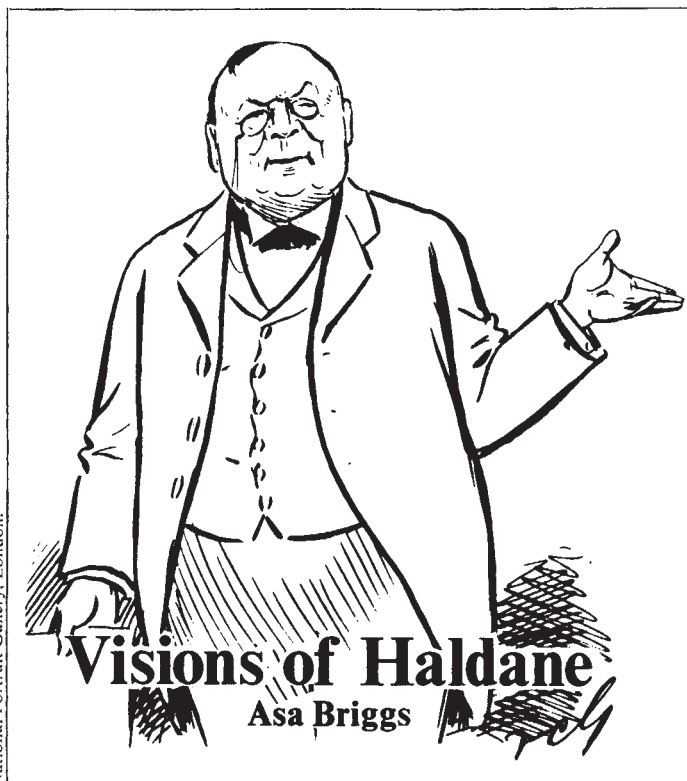
No one emphasised more than he the need for 'system' in education, yet he conceived of that system in organic and not in mechanical fashion: everything educational was part of the same "organic whole", from nursery schools to Workers Educational Association classes. It followed from this approach that universities were not separate

chose his arguments to suit his audiences, would have been unsuccessful had he not been an indefatigable as well as a clever manipulator and, above all, "a great public personage".

Ashby and Anderson explain well how his approach, shaped by German philosophy and operated through well selected manoeuvres, affected the emergence or development of

particular institutions—among them Imperial College and the University of Liverpool—and of the University Grants Committee as the guardian of the 'system'. Their conclusion is well balanced. They recognise that even without Haldane's personal contribution, much of what has subsequently happened in higher education in this country would have happened anyway. Yet without him, they add, "the style and tenor" of what has happened would have been different and "poorer". His conceptions were not original, but through his own efforts they were "institutionalised". Of course, this only applies to some of them. When the Robbins Committee turned to structures in higher education years later, it referred to "a system if it can be called a system". The 'binary system' Haldane would have deplored—though all its outlines were there before he died—and there is still no 'system', even in an embryonic form, in adult education in spite of the valiant efforts not only of Haldane but also of Ashby.

It is possible to discern in the well organised pages of this biography sketches of alternative notions of education, deliberately less systematic or even non-systematic, for Haldane



institutions with their own vested interests but necessary elements—differentiated perhaps in styles—of 'national education', the development of which was successful "in proportion as it is guided by a policy which regards it in all its bearings and aims at settled objects". In practice, few educational institutions, except new ones, have ever approached national education quite in this way, and Haldane himself, who

always had his critics. He once wrote to Joseph Chamberlain mentioning "Oxford notions of what a University ought to be", and these would certainly have to be taken into account if this portrait of Haldane were to be expanded into a monograph on continuity and change in British higher education. The very idea of 'system' offended some critics, and the German philosophical language in which it was often expressed (sometimes with dangerous political potential) offended more.

Given a systematic approach in a country where it is easy to spurn enthusiasts in education as well as logic, what obviously counted then, as now, was the quality of the people making the key decisions concerning its operations and planning. Haldane was fortunate that there were far-sighted civil servants in London who shared his vision, but what would have happened had his ideas on 'regionalisation'—Ashby and Anderson call them a gospel—been fully implemented? Those are ideas which are not yet dead, although they are often advanced by people without vision or Hegelian philosophy—politicians and others seeking to co-ordinate in order to make education cheaper or "more equal". It would have been interesting had Ashby and Anderson pursued this story a little further, for they do not return to it in their epilogue. Haldane envisaged a system of 'regional' universities—and this involved bringing new universities into existence—each with responsibilities outside higher education as well as in extramural studies.

He never doubted, however, that the universities should "preside benignly" over the regional sub-systems which he called "educational provinces". The "great kind of coordination" of which

he dreamed was not achieved, but if it had been pushed further would it have been under the aegis of the universities? Haldane did not envisage the local authorities as the providers of funds—though he insisted that they should have an initiating and maintaining role in the dynamics of development—but even without funding powers would they have been willing to allow universities "the powers necessary to enable them to organise education from top to toe in their own districts"? How many of them, given expansion of student numbers, would have shared Haldane's view that higher education was "an end in itself"? How many members of his own party shared this view at the time?

Many of the most valuable paragraphs in this book are concerned with aspects of British higher education which do not relate exclusively to Haldane but which have never been very carefully considered in historical perspective. Thus, the sections on the University Grants Committee dispose of some historical misunderstandings and there are new insights into Fisher's Education Act of 1918. What often stands out is the meanness of the Treasury; a few thousand pounds can be argued about inordinately. It is sad to hear Haldane making remarks like "the only effective way of getting the money is by private appeals to very rich men", but it is sadder still to note that, leaving the Treasury on one side, very few rich men indeed showed the slightest interest in responding.

Portrait of Haldane at Work on Education. By Eric Ashby and Mary Anderson. Pp. xvi+202. (Macmillan: London and Basingstoke, November 1974.) £5.95.

To cross linguistic barriers

After Babel: Aspects of Language and Translation. By George Steiner. Pp. viii+507. (Oxford University Press: London, New York and Toronto, January 1975.) £8.00.

ONE strain in the extraordinary logical and philosophical ferment of the Thirties was I. A. Richards' thesis that literature itself had become a proper subject for scientific investigation in that general hypotheses about meaning and interpretation could be tested by the examination of texts. George Steiner's magnificent book should be seen as a survivor from that age of dinosaurs, still very much alive in our own time. To read the book one must actually be interested in literature; it is not enough to be schooled in any of the more recent attempts to handle language in a scientifically acceptable manner, such as Chomskyan linguistics or artificial intelligence.

In Steiner's view, the scientific approach to language has got into the hands of the bad guys, of people who have forgotten what language is actually like. To the extent to which he is right, the book should be compulsory reading for 'language scientists'. He draws attention to the fact that they are usually monolingual (he has been trilingual from an early age) and that little that they have to say is relevant to the real world of spoken and written language because they have a false picture in that they see language simply as a projection of whatever formalism they happen to subscribe to, a formalism that may be quite untrue to the language that comes out of their mouths, just as earlier theorists speculated about phlogiston while their lungs kept them alive by absorbing oxygen.

One might reply that criticism of a scientific activity by appeal to cultural information is absurd: Galileo's opposition, after all, had strong literary evidence for the movement of the Sun. But with language the case is different and, unlike Koestler, Steiner is taking on not established sciences, but would-be sciences like linguistics, philosophy and artificial intelligence; subjects on which Steiner is as much entitled to a hearing as the next man. In particular, he is arguing that anything claiming to be a theory of language must have something interesting to say about translation.

There are three obvious troubles with the way Steiner makes his case: his style of argument, the implausibility of his central thesis about the equivalence of translation and interpretation, and the omission of a large issue that scientists would like to see raised (and

THE brain is a mass of soft matter, in part of a white colour, and generally striated; in part of a grey or cineritious colour, having no fibrous appearance. It has grand divisions and subdivisions: and as the forms exist before the solid bone incloses the brain; and as the distinctions of parts are equally observable in animals whose brain is surrounded with fluid, they evidently are not accidental, but are a consequence of internal structure; or in other words they have a correspondence with distinctions in the uses of the parts of the brain.

On examining the grand divisions of the brain we are forced to admit that there are four brains. For the brain is divided longitudinally by a deep fissure; and the line of distinction can even be traced where the sides are united in substance. Whatever we observe on one side has a corresponding part on the other; and an exact resemblance and symmetry is preserved in all the lateral divisions of the brain. And so, if we take the proof of anatomy, we must admit that as the nerves are double, and the organs of sense double, so is the brain double; and every sensation conveyed to the brain is conveyed to the two lateral parts; and the operations performed must be done in both lateral portions at the same moment.

I speak of the lateral divisions of the brain being distinct brains combined in function, in order the more strongly to mark the distinction betwixt the anterior and posterior grand divisions. Betwixt the lateral parts there is a strict resemblance in form and substance: each principal part is united by transverse tracts of medullary matter; and

The observations of Charles Bell. From *The Way In and The Way Out*. By Paul F. Crane. (Futura: New York, 1974.) \$25.00.

almost every other issue one can think of is raised), namely scientific language. I shall look at these in turn.

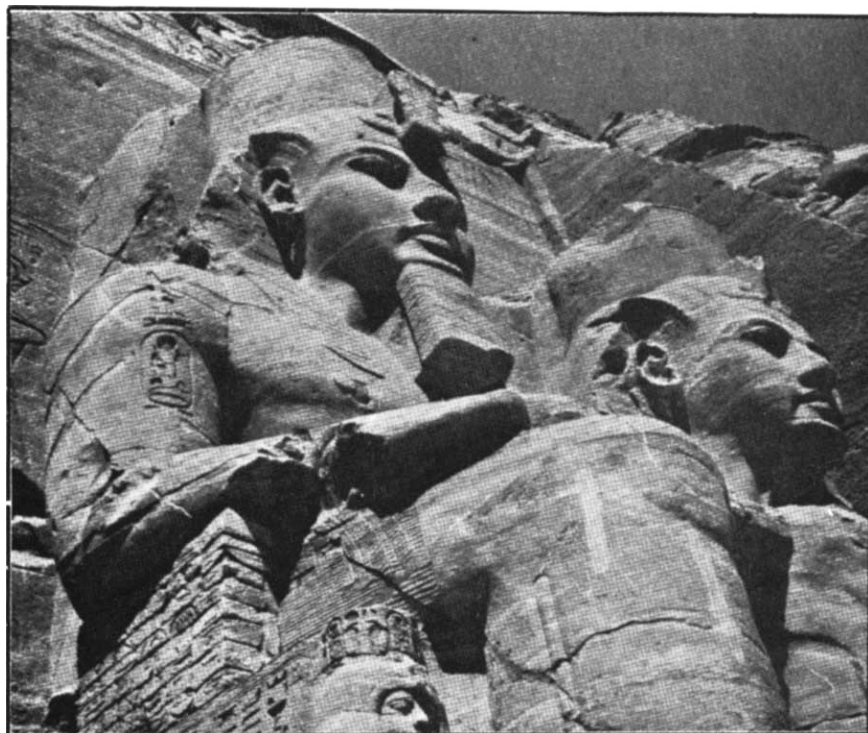
The central theme is the curse of Babel: the diversity of human languages; and the central thesis of the book is that every act of communication requires an interpretation, and that an interpretation is essentially a translation, whether across a language barrier, across a time barrier (as when we attempt to interpret Shakespeare) or across a barrier between individuals or groups. The book opens with a long speech from *Cymbeline*, and Steiner asks in detail what it means to us now. He turns to other passages in similar detail, ending with Noel Coward, always making the same point: a proper interpretation is hard, even at only 40 years' distance, but with the diligent application of critical method it can be done.

Before I confront his thesis briefly, a digression on the style of the book is essential. Under six chapter headings, Steiner raises an enormous number of issues, sometimes more than one on a page: does language determine our actual perception, as Whorf believed; what was Leibniz's view of a universal syntax and how does it relate to Chomsky's; is women's grammar different from men's; what effect had the statement of the second law of thermodynamics on sensibility and speech at large?

These topics are all raised and dropped during nimble and ebullient leaps from name to name and epigram to epigram: "History is a speech-act, a selective use of the past tense"; "Though the great master Tartakower thought otherwise, we do not ascribe feelings . . . to chess pieces"; "But for all their lively truth, children in the novels of James and Dostoevsky remain in large measure miniature adults. They exhibit the uncanny percipience of the 'aged' infant Christ in Flemish art. Mark Twain's transcriptions of the secret and public idiom of childhood penetrate much farther"; and even, "Sex is a profoundly semantic act".

Something intriguing is almost always being said, but it is seldom developed to a point of clarity, or to where the weight of literary evidence, familiar and arcane, that Steiner marshals can tell against his claims and throwaway remarks in any definite way. For there is no time to analyse and discuss in any depth as the whole vast parade of central European scholarship moves on, usually just at the point where one longs for detailed analysis and more examples.

Even what I called the central thesis is not discussed directly in the way it deserves, so let us return to that for a moment. The point of the thesis is to blur the distinction between interpreta-



The Colossi of Ramses at Abu Simbel, guarding the entrance to a temple penetrating 45 metres into solid rock. Possible evidence that the now barren Nubian desert was once comparatively fertile. From *The Ecology of Oases*. By J. L. Cloudsley-Thompson. Pp. vi+43. (Marrow: Watford, 1974.) £1.25; \$4.40.

tion and translation: between the way an individual confronts his own language and the way different languages confront one another. Steiner gives too much weight to what one might call the Telex view of human understanding: that we sit, as it were, in our private offices trying to interpret language coming in from outside. This view makes human communication seem a problem, and is a recrudescence of an old-fashioned empiricism, one that ignores the essentially performative and social nature of language.

If one rejects the 'Telex view' there seems an essential difference between trying to understand what another speaker of your own language is saying, and doing the same for a different language. In the case of interpretation we have the ability to go on asking someone what he means, to form hypotheses, as it were, and test them in discussion. In the case of translation that is exactly what we do not have, and thus the distinctive problems of translation arise. We are, in general, deprived of just that essential "further elucidation" aspect of language.

Steiner's key examples are of interpreting one's language diachronically: he argues that any English of the past is another language, and similarly for the language of women, of the upper classes, and so on. The heart of the matter is the elusive Wittgensteinian notion of a "form of life": in that we can interpret/translate only if we share

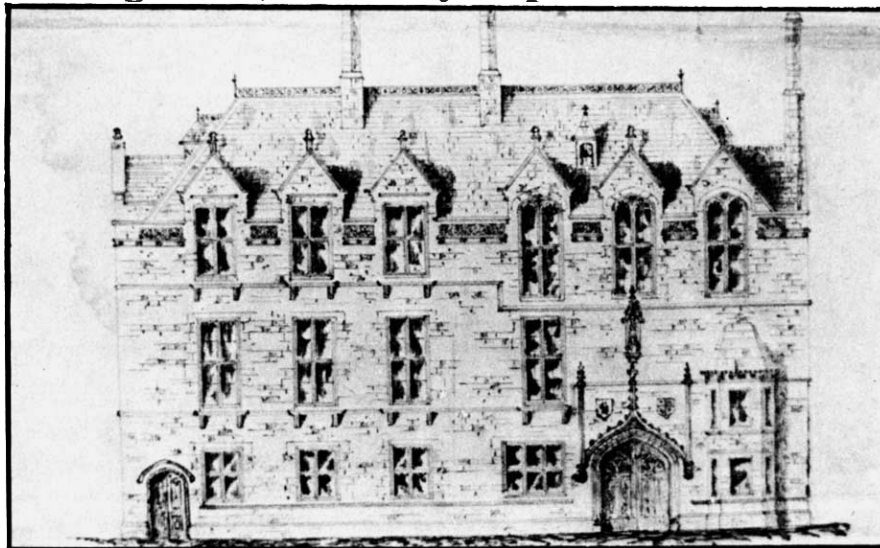
an adequately close form of life with those who are the source of the language. One might say dreamily that it is this background knowledge that workers in artificial intelligence are at present trying to formalise and put into their programs.

A large topic presents itself here that it would have been nice to see Steiner discuss: namely, is science such a form of life, and if so can scientific language transcend other cultural boundaries? The question should have come up in the extended discussion of Whorf and his thesis that different cultures have languages describing essentially different worlds. Steiner might well have brought out that Whorf explicitly extended this thesis to scientific language and argued that a psychologist, physiologist and physicist describing, say, the same brain, are speaking different, not mutually translatable, languages and, in that sense, have different cultures. This seems implausible, for we feel that science is a uniform international culture and language. And yet, had Steiner found room for this topic he would almost certainly have drawn our attention to modern Chinese scientific journals, particularly medical ones, where it is not at all clear that they can be understood outside the Maoist frame of thought.

It is a highly personal, stimulating and infuriating work but, warts and all, a considerable achievement.

Yorick Wilks

Solid ground, Himalayan peaks



Cambridge Evening News

The Cavendish Laboratory, 1874–1974. By J. G. Crowther. Pp. xvi+464. (Macmillan: London and Basingstoke, October 1974.) £25.00.

THE foundation of the Cavendish Laboratory a century ago was the greatest single consequence of the mid-Victorian movement to advance the cause of science in Britain. During the preceding two centuries the development of science in the English universities had been paralysed by the Clarendon Code which, as G. M. Trevelyan said, subjected them to “the bondage of Church monopoly” so that only professing Anglicans could teach and study there. Most science in England was therefore found outside the universities—in the Lunar Society at Birmingham, or the Literary and Philosophical Society at Manchester, or the Royal Institution—a fact from which Cardinal Newman ingeniously argued that experience had shown that for its own well-being science should be pursued outside universities rather than within them. Only in Scotland, where the Clarendon Code had been repealed following the 1688 Revolution was it possible for non-Anglicans and free thinkers to go to university—a fact which contributed much to the eminence of Scottish science, philosophy, and economics in the century and a half before 1871, when at last the University Test Act abolished religious discrimination in England.

In the same year James Clerk Maxwell was appointed as the first Professor of Experimental Physics in Cambridge. The university had procrastinated for some years over the creation of the Chair and its associated laboratory, having been influenced by such remarkable arguments as “A Prussian is a Prussian and an Englishman an Englishman and God forbid that it

should be otherwise” by the Master of Sidney Sussex. What finally moved the university was the offer (which was accepted) by its Chancellor, the Duke of Devonshire, to cover the cost of building the laboratory and of buying its equipment.

In this history, Mr Crowther sets the foundation of the laboratory in perspective, and takes us through its continuously happy fortunes ever since. No Chair in the world has had such a distinguished and creative a succession of holders, and Mr Crowther provides many sidelights on their personalities. We find Maxwell, for example, working in his shirtsleeves with each hand in a basin of water to compare the strengths of electric currents, and exclaiming “each man his own galvanometer!” by way of explanation to an American visitor, S. P. Langley (of the bolometer and flying machine), who had been brought unexpectedly by Schuster to be introduced.

Maxwell at the time was confirming the experiments of Henry Cavendish, whose papers he edited in what Mr Crowther describes as “the finest contribution to the history of science in the English language”. Maxwell had a strong historical sense but Mr Crowther makes the general point that “The Cavendish, like the whole of British science, was sublimely disinterested in its historical aspects. Why British science should have been so non-historical is a significant question”. Perhaps it is a sign of exhilarating progress to have so much to do and think about in the present and the future that effort in attending to the past, or even preserving the present for the future, is grudged. But it has resulted in a cleavage in Britain between practising scientists and those who style themselves “professional historians of science” that too few of us are trying

to repair, and it is a pleasure to acknowledge Mr Crowther’s splendid efforts over many years in this direction.

Among the many illuminating comments that he has gathered from past and present Cavendish members there is one that—if it really means what it says—is mildly surprising: “Some consider that the Cambridge undergraduate . . . has done more for Cavendish science than the talented research students coming from outside. They produced a large body of very good scientists of the second level, which provided an excellent support for the front rank of genius”. Such a comment must have overlooked the fact that J. J. Thomson, Rutherford, Chadwick, Aston, C. T. R. Wilson, Cockcroft, Ryle, and Crick were all undergraduates elsewhere; and Maxwell took his first degree at Edinburgh.

Besides its value as straightforward history there is much to be dug out of the book by anyone interested in the problems of running a laboratory and of seeing it through from one phase of success to another. There is, for example, the principle that Bragg took over from military organisation that one man cannot effectively keep contact with more than six subordinates on a day-to-day basis, which led to the organisation of the large laboratory into groups which in turn may have again to be sub-divided. And those who press for the centralisation of university services such as workshops might be reminded of the experience that, even inside a single laboratory such as the Cavendish, “It was much more economical to do as much as possible in a small workshop belonging to a group rather than in a central workshop”. And again there is Bragg’s estimate that Britain produced one good physicist a year per million inhabitants—a sobering reflection on the optimism of the Robbins expansion.

The one truly unfortunate thing about the book is its price—£25.00 after the publishers had already advertised it at the prohibitive price of £14.00. And especially in view of the price the quality of the photographic reproductions is poor. For neither of these defects is Mr Crowther responsible, and it is much to be hoped that somehow the story which he tells will be made much more widely available than the present price allows. For the story is magnificent: Maxwell and Rayleigh setting the foundations; J.J. discovering the electron (when the total bill for 1896 was £1,067 7s 0d plus his own salary and another £420 for the salaries of university lecturers and demonstrators); C.T.R. inventing the Wilson Chamber; Aston finding the mass defect and thereby the clue to nuclear energy; Cockcroft and

Walton transmuting lithium with protons; Chadwick finding the neutron; Blackett recognising the positive electron; Crick and Watson elucidating the structure of DNA; Josephson predicting his effect; Hewish and Jocelyn Bell finding pulsars. Besides this Himalayan chain of peaks of discovery in the Cavendish itself there have been major discoveries by Cavendish men working elsewhere—for example, the wave nature of the electron by G. P. Thomson or the π -meson by C. F. Powell. But the Cavendish story is not just of these peaks; there has been an enormous amount of supporting work over a wide range of physics, and from the Cavendish many physicists have gone outside to other universities, to the schools, to industry, and to government establishments.

So many achievements could induce complacency; but so long as the Laboratory maintains the spirit of Professor Pippard's 'The Cavendish Tradition' (*Nature*, **249**, 602–603; 1974) it need have no fear that "Nothing fails like success".

R. V. Jones

Scientists only human in their quest

Originality and Competition in Science: A Study of the British High Energy Physics Community. By Jerry Gaston. Pp. xix+210. (University of Chicago Press: Chicago and London, 1974.) £5.50.

GASTON'S sociological study of the British high energy physics (HEP) community is most interesting. Knowledge in the natural sciences with all its fallibilities is less uncertain and less subjective than knowledge in the social sciences or the humanities. The corollary has, too often, been a belief that natural scientists conduct their scholarly inquiry in a more rarefied way than other people and are indeed rather more rarefied people. This belief has been encouraged by the historians and philosophers of science and sociologists have added their norms. Dr Gaston quotes four norms or rules which Robert Merton ascribes to scientific activity: organised scepticism, universalism, communalism and disin-

terestedness. Each norm is not unique to science but together they are said to be peculiar to the ethos of modern science. American sociologists of science, led by Merton himself and by scholars such as Hagstrom have, however, shown how often scientific activity diverges from those norms.

Dr Gaston looks at some of the norms in relation to the British HEP community at one point in time—1967–68. One of the advantages of his study is that its coverage is almost complete and does not rely on small samples: about 220 scientists were located at 23 different universities or government establishments, of whom 203 provided information and were interviewed.

The author has analysed their social, educational and professional background; the kinds of research they conducted; their contributions to the scientific community—that is, their productivity; the recognition they received from the scientific community; their attitudes towards research; the prevalence and severity of competition; and their communicative behaviour. Throughout the book, in pursuing these themes, Gaston distinguishes between the views and habits of theoretical and experimental physicists.

He also makes valuable comparisons with the studies of American scientific communities. It is, however, unfortunate that he follows too closely the pathways set by his predecessors so that some of the problems which are especially interesting in HEP are omitted. Thus, he omits the scientists who operate accelerators and other machines "because they are more akin to engineers than scientists". He does not mention the machine builders at all, but it is wrong to dismiss the machine-providers whose performance in terms of sheer physics alone has been so great. The relationship between them and the machine-users is important in the study of any HEP community. Again, in high energy physics, the identity of the individual scientist has increasingly been lost within team identity. Much more attention needs to be given to the effects of this change in the property system of science upon motivation and relationships.

These qualifications apart, Gaston produces 45 statistical tables which reveal fascinating positive or negative correlations. For example, why are high energy physicists significantly more productive at some institutions than at others? How is the number of publications related to prestige of the university at which the PhD was gained, prestige of present affiliation,

The Ciba Foundation: An Analytic History, 1949–1974. By F. Peter Woodford. Pp. viii+212+8 plates. (Associated Scientific: Amsterdam, Oxford and New York, 1974.) Dfl. 38; \$14.75.

THE decision of the Ciba Foundation to commission an analytical history of its first 25 years has turned out to be a wise venture. The analysis, undertaken by Dr Peter Woodford, has been carefully performed and, through his avowed aim to be critical, he has managed to bring out a number of points of importance. The Ciba Foundation with its symposia and publications is so well known that it is in danger of moving beyond the range of criticism. It is, therefore, of importance to analyse the secret of its success and to try and separate the method from the qualities of those who run it. Inevitably, that is an impossible task.

The Ciba Foundation is a product of the knowledge, experience and attitudes of Dr Gordon Wolstenholme and his able colleagues. The fact that their purpose and method of working was timely and has filled a special place in the world of medical research is a further tribute. Maybe all our institutions are a product of individual effort at a special time. Certainly, it is always very difficult for any successor to take over and continue where his predecessor left off and this will be equally true of the Ciba Foundation because of the unique, accumulated interpersonal relationships developed by its staff.

The importance of this book lies, therefore, in its analysis of those features of the foundation that may be greater than its leaders. The analysis of the reasoning which led to the decisions on the size and nature of the meetings in general and also for more specific topics is thus the most important aspect of the book.

It is also important that a study has been made of the impact on medical research of the activities of the foundation. This has proved to be a difficult task which has daunted many who have previously attempted it in other organisations. Sufficient to say that the record of the Ciba illustrates the flow of progress that can develop from bringing together, in relaxed surroundings, people from different disciplines and nations so that they may stop for a while to contemplate matter that are set on one side in the busy working day. The Ciba Foundation has achieved its aim and provides the ideal illustration of the importance of having specialised activities supported by private funds. It also shows that it is not size, but quality, that counts.

I can make only one factual criticism. It is not correct to say (page 4), "The essence of the law (Charities Act) is that those who provide the money disposed of by the trust may not be represented on its governing body". This book will be of interest to all those who know and admire the Ciba Foundation through its books or meetings. It is a worthy tribute to the singleness of purpose of Gordon Wolstenholme.

P. O. Williams

proportion of time spent on research, number of hours spent weekly on research, proportion of time spent on teaching, class of undergraduate degree, chronological age, professional age? What is the relationship between recognition and these variables and what is the relationship between productivity and recognition? Competition is measured by the number of times scientists have been anticipated through having research findings similar to their own published first by someone else. What anticipation have British HEP workers experienced, for example in relation to their rank or by comparison with American scientists? Having been anticipated, did they publish or not publish? As for communication, what is the most important method of obtaining information relevant to research, by type of HEP, rank, professional age?

How does verbal communication work and which are the most important journals?

Equally, if not more, illuminating are the extracts from transcripts of conversations about competition. One suggestion is that competition helps to motivate scientists who otherwise might put forward less than their best effort. An experimentalist is quoted as saying, "I have suspicions that less than one physicist in three that I've met is really and deeply and drastically motivated by a desire to 'find out'". There is much material in this book to show just how cut-throat the competition can become—both between individuals and between continents—together with material which suggests why American physicists 'beat' European physicists in certain races of discovery. Gaston records his evidence about the "three main

methods of deviance" which scientists sometimes use to get the recognition which eludes them: hasty publication; fraud and theft; and secrecy.

In his conclusions, the author does not ask squarely where his own evidence leaves Merton's four rules of science. He finds that the reward system in British HEP communities, unlike the system in America, operates in a universalistic fashion. Recognition is highly related to productivity and theorists receive more recognition than experimentalists. But affiliation with a particular type of university or a department in a particular prestige category has no effect on opportunities for scientific recognition. The four rules are, however, meant to hang together and Gaston shows that scientists are indeed human in their quest.

Margaret Gowing

The Competent Infant: Research and Commentary. Edited by Joseph L. Stone, Henrietta T. Smith and Lois B. Murphy. Pp. viii+1,314. (Tavistock: London, 1974.) £11.00.

Most books consisting of collections of published papers are worth neither reviewing, buying nor reading: the present volume is an exception. The editors have done a remarkable job of arranging the selected papers in logical order, shortening them, and with the help of their own commentaries producing a coherent book. There are two further reasons why this collection is particularly useful. First, infant development is a topic on which spectacular progress has recently been made and second, as there are few journals devoted specifically to it, papers on the subject have appeared in such a wide range of journals that no one person could possibly scan them all: in *The Competent Infant*, we find papers from the *Journal of Ophthalmology* rubbing shoulders with extracts from *Psychosomatic Medicine*, *Vita Humana*, and *Cognitive Psychology*.

The book demonstrates the ways in which our ideas about the competence of infants have been revised drastically by the work of such pioneers as Wolff, Escalona and Prechtl. Until some 20 years ago, it was widely accepted that new-born babies were almost entirely passive and incapable of anything much in the way of organised adaptive behaviour. More recent research shows quite the contrary and the watershed may be placed at the publication of Wolff's now classic paper in 1959. He found that, if you catch your infant at the right moment, it will emit highly organised and complex behaviour. As Prechtl puts it: "If you put a new-born infant baby in a supine position

in its cot and cover it with a blanket up to the neck, of course it gives the impression of being a kind of vegetable which just cries and sucks from time to time and that's all". What Wolff discovered was that the reactivity of the new born depends on its state. He classified such states into sleep (regular, periodic and irregular), drowsiness, alert activity and alert inactivity. It is only when the infant is alert and

behaviour tends to occur immediately after the baby has satisfied its physiological needs such as feeding and defecating. Experimenters patient enough to wait for such periods have been rewarded by observing complex behaviour even on the first day of life: some infants in fact show about an hour of alert inactivity starting 15 minutes after birth.

Recent research, stemming from Wolff's insights, has shown that there is considerable innate organisation of perceptual and motor functions. One of the most remarkable experiments republished in the present book is Sackett's ingenious demonstration that monkeys have an innate capacity to recognise not merely other monkeys but whether or not the other monkey is in a threat posture; this work is now nearly 10 years old and its implications are so important that it is very regrettable that, as far as I know, there have been no independent attempts to replicate it. There is also ample evidence that personality traits which tend to remain constant throughout life may appear in new-born infants: American babies of Chinese origin show less irritability and impatience than do whites born on the same ward and tested within a few hours of birth.

The book should give pause to those who think either that psychologists rarely discover anything we did not know already or that introspection and every day experience are all we need use to infer the structure of the mind—a belief that has recently been resuscitated by some polemicists for the new branch of technology known as 'artificial intelligence'.

The book is 1,300 pages long and nicely produced: at £11.00 it surely rates as a best buy. N. S. Sutherland

More than vegetables



inactive that it will attend to outside stimuli; when it is alert and active, crying or thrashing around, it pays little attention to external stimuli, particularly to new stimuli. During the first week of life, the infant spends only about 10% of the day time in alert inactivity. In this state "his respirations are regular . . . his eyes are wide open, shiny and capable of conjugate eye movements, and he makes visual and auditory pursuit movements . . . the skin is pink but not flushed". Such

More behaviourism

About Behaviourism. By B. F. Skinner. Pp. 256+viii. (Jonathan Cape: London, February 1975.) £3.50.

PROFESSOR Skinner is unrepentant. Assailed by the liberal and radical establishment as a reactionary witch-doctor intent on subjecting human freedom to blind conditioning, attacked by the new school of linguistics and cognitive epistemology as an ignorant primitivist, Skinner not only maintains his position but makes it more dogmatic.

The concept of mind is an obscurantist 'invention' largely attributable to Plato. The only distinctive characteristic of verbal behaviour is the fact that "it is reinforced by its effects on people". Dreams, fantasies, *déjà-vu* are merely re-cognitions of "what we have once cognised", in some of which certain controlling features of self-knowledge are defective. Human thought equals human behaviour: *sum ergo cogito*. Faith is just "a matter of the strength of behaviour resulting from contingencies which have not been analysed". Mentalist explanations "explain nothing". Behaviourism, on the contrary, puts us in a position to alter and improve the condition of mankind if only we will apply its commanding insights into the role of the environment and into the agency of such cardinal notions as reinforcement and operant behaviour.

Neither the strategy of the argument nor the tone of mildly outraged common sense are at all new. If there is innovation it can be found in the tactics of Professor Skinner's case, particularly in his increasing resort to a Darwinian idiom. In an important sense, we are told, "all behaviour is inherited, since the organism that behaves is the product of natural selection. Operant conditioning is as much a part of the genetic endowment as digestion or gestation". The origin of behaviour "is not unlike the origin of species. New combinations of stimuli appear in new settings, and responses which describe them may never have been made by the speaker before, or heard or read by him in the speech of others. There are many behavioural processes generating 'mutations', which are then subject to the selective action of contingencies of reinforcement."

What is going on here is plain. Darwinian and post-Darwinian terminology is being used in an attempt to refute the drastic onslaught on behaviourism by Chomsky (whom Skinner, either in coyness or contempt refuses even to mention by name). Dismissing the entire basis of innateness in transformational-generative theories of speech and consciousness, Skinner

declares that "grammatical behaviour" has always been shaped by the reinforcing practices of given verbal communities in which some behaviours were more effective, more provocative of useful response, than others. Sentences are not generated by imaginary 'deep structures' but, like all other human proceedings, by "the joint action of past reinforcements and current settings".

As one follows this controversy, a sense of exasperation mounts. It is so utterly clear that both sides simplify, distort, and finally trivialise reality. Skinner is perfectly right when he observes that Chomsky's model is full of logical and evidential loopholes, that it absurdly neglects environmental-historical factors, that it is dogmatic and even 'mystical' in its presupposition of deep structures and mental 'pre-settings'. Chomsky, on the other hand, has shown beyond doubt that a stimulus-response paradigm along Skinnerian lines is totally inadequate to explain either the process of language acquisition and innovation or the prevalence (or what looks like the prevalence) of rule-governed similarities across the field of all known grammars. But, surely, there is no need here of a doctrinal either/or. The evolution of human speech and cognition is neither Skinnerian nor Chomskyan, but represents a constant dynamic reciprocity between universal neurophysiological features on the one hand and the contingencies of the environment on the other, between predisposition and reinforcement, between mimesis and discovery. Indeed, using a Darwinian analogue, one would want to argue, with far more attention to the facts than is shown by either behaviourism

or transformational-generative linguistics, that man's language capacity is uniquely adaptive. Through words he constructs worlds more bearable than that of his organic surrounding. Through the future tense he does something to circumvent the totality of biological death.

Skinner's contributions to experimental psychology, to learning theory and the understanding of conditioning and reinforcements stand secure. It is a pity that they should be overshadowed by an embattled dogmatism the political add philosophical consequences of which are not necessarily sinister, but are certainly naive. **George Steiner**

Animal Nature and Human Nature. By W. H. Thorpe. Pp. xviii+435. (Methuen: London, January 1975.) £7.20.

It is of interest to compare *Animal Nature and Human Nature* with Skinner's new book, *About Behaviourism*. Although Thorpe thinks human behaviour cannot be explained solely by genetic and experiential factors whereas Skinner adopts a strictly deterministic position, the similarities between the two books are more striking than the differences. Both authors believe that if human behaviour were so determined, our freedom of will would be constrained; both rely heavily on assertion and on appeals to authority rather than on argument, though naturally the quoted authorities differ; both dogmatically reject computer programs as possible models of the mind without examining any such programs; both put forward Utopian views of man's future without taking the trouble to think out what sort of Utopia they want; neither gives evidence of having read, let alone



All slots open, flaps down, and undercarriage lowered, the barn owl approaches home. From *Natural History Photography*. Edited by D. M. Turner Ettlinger. Pp. xxvii+395. (Academic: London, 1974.) £8.80; \$23.25.

understood, any arguments against their own position; finally, nothing of any scientific or ethical consequence follows from the contrasting stances taken up by each author.

Thorpe's book is perhaps the less bad, if only because, unlike Skinner, he gives some interesting examples of the results of his own science. He culls from recent work in ethology instances of fixed action patterns, animal communication systems, and social organisation in mammals. Such phenomena have an inherent fascination: like most ethologists Thorpe is at his best when describing such behaviour and speculating on its survival value. He is less good at specifying the mechanisms that mediate it. On many issues, such as nature against nurture, he takes a sensible position—he rightly castigates Hebb for his declaration that it is absurd to ask how far a given piece of behaviour is innate, how far it is learned. Thorpe's chapter on perception is weak: he still thinks of perception in terms of pattern recognition and fails to get across the idea that perception must involve forming representations of the outside world which can be manipulated in ways that serve to guide the organism around. In this chapter, his accounts of much experimental work are somewhat cursory and inaccurate.

In arguing for an antireductionist, emergent view of mind he fields the standard team of obscurantists: Koestler, Polyani, McKay, Eccles, Dobzhansky, Weiss, Hardy and Teilhard de Chardin. His eleven is made up by enrolling two new players, Chomsky and Popper, and by using a theologian (Hick) as long-stop. With the exception of the two beginners, these players proceed to throw around terms like 'emergent', 'consciousness', 'determined', 'free-will', 'wholes-greater-than-the-sum-of-their-parts', and so on. We are treated to such sentences as "the ego's view of the physical world will thus be mediated by a mechanism working just like television". On page 341 there is even a diagram showing (with a dotted line) how "mental space" intersects three-dimensional physical space. The players have such an enjoyable time tossing the ball to one another and cheering each other on, that they seem quite to have forgotten to notice the opposition. Nevertheless, they make their opponents' task very difficult, since the game is played with no rules or definitions; but it may be worth examining a few of the many *non sequiturs*.

Thorpe accepts McKay's proof that behaviour is indeterminate: in attempting to predict our own behaviour, we change our internal state and, short of an infinite regress, we cannot take such changes into account in making our

predictions. The premise is correct but the conclusion does not follow: do we regard computers as being indeterminate because no program could completely predict what it will do? Again, it is correctly stated, following Popper, that at each level of explanation it is necessary to evolve concepts appropriate to that level. To explain and predict the behaviour of a gas we need concepts like pressure, appropriate to collections of molecules not to individual molecules, and to explain the workings of a computer program we need concepts such as 'conditional jump', 'recursion', and 'iterative loop' that cannot be applied to transistors. If this is all Thorpe means by "emergent qualities", then we can readily concede that a system with the highly unusual organisation of the brain is likely to have emergent qualities, without committing ourselves to the belief that there is some special sense in which human behaviour is 'undetermined' and without supposing that there is a two-way interaction between consciousness and matter. Thorpe also uses the "gawping at Nature" argument: we do not understand why some bird songs and displays should be as elaborate as they are, but present ignorance is no reason for assuming that no scientific explanation will in future be found. It is particularly curious that Thorpe should use this argument since else-

where he points out that some highly complex behaviour once thought to indicate the presence of a life-force or creator can nowadays be explained in mechanistic terms.

The assertion that "Sir Karl Popper comes down with all the weight of his great learning and experience heavily on the side of dualism" surely does not exempt Thorpe from considering the difficulties inherent in this position. The polemical parts of the book are written in the vaguest of terms. Thorpe dismisses computers as models of the mind without discussing any work in artificial intelligence, yet telepathy and clairvoyance are uncritically accepted on the strength of the questionable work of such experimenters as Soal and of Koestler's collection of unlikely coincidences. The distinguishing characteristic of the human race is said to be religion.

The book is based on a series of Gifford lectures delivered at St Andrews: such lecturers are instructed, under Lord Gifford's will, to deal with natural religion "as a strictly natural science, the greatest of all sciences"; but this instruction is usually interpreted by the lecturers themselves to mean they should treat of their own subject matter in religious terms. The present book is a fine example of the Gifford Lectureship Syndrome.

N. S. Sutherland

Factors leading to schizophrenia

Genetics, Environment and Psychopathology. (North Holland Research Series on Early Detection and Prevention of Behaviour Disorders, vol. 1.) Edited by S. A. Mednick, F. Schulsinger, J. Higgins and B. Bell. Pp. xiv+346. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1974.) £9.25.

IN 1962 the Psykologisk Institut in Copenhagen began a research project aimed at the early detection and prevention of mental illness. This book brings together a number of studies conducted at the Institute over the last 10 years. Many have contributed to the research, and 20 authors from the USA, Denmark, Iran and the UK, write in this volume.

The authors were dissatisfied with research on people already schizophrenic and so they conducted prospective studies of people at risk. They used children of schizophrenic parents and selected control groups. Unexpectedly, they found that perinatal factors influenced later outcome and so they established a new study in greater detail, using babies born in Copenhagen between 1959 and 1961. They linked that with Professor Preben Plum's detailed peri-

natal study of 9,006 deliveries during that period.

The methods that were used are described fully. They were of a high standard and good contact was maintained with family doctors and hospitals. There is a full list of references which will be valuable to others studying in this field, and there is an index.

The book represents a considerable advance towards the unravelling of genetic and environmental factors in the origins of schizophrenia. Electrodermal responses were used in several of the studies, and some of the authors characterise schizophrenia as a learned pattern of avoidance responses. As treatment seems to offer little, attention has been concentrated on methods of prevention of the development of schizophrenia in 'high risk' children. The authors have made excellent use of the Danish Register of Adopted Children. Schizophrenia is only one of the mental illnesses studied, and the effect on being reared by a schizophrenic mother is tentatively separated from inherited factors.

This volume is good value for money for all research workers in this field and will be of great interest to all field workers.

R. Mac Keith

Evolution of sex

THIS is an important and infuriating book*. It is important because it is a scholarly and sustained attack on the most important problems in evolutionary biology; the evolution of genetic mechanisms, of sexual dimorphism and of society. It is infuriating because it fails, narrowly but decisively, to provide the illumination promised by the first two chapters.

The problem which Ghiselin sets out to cover is more easily stated than solved. In Darwin's theory of natural selection we have an explanation of evolution in terms of the success or failure of individuals to survive and leave descendants. As an explanation of characteristics which promote individual survival and reproduction, this is fully satisfactory. But sex and genetic recombination are often interpreted in terms of their contribution to the survival of the species and not of the individual, and social behaviour in terms of the survival of the community. How is the evolution of such characteristics to be explained? Two positions seem possible. The first, the "group selection" explanation, is to argue that selection between species (that is, the survival and division of some species and the extinction of others) produces genetic mechanisms adapted to ensure species survival. The second, strongly espoused by Ghiselin, is that sexual adaptations evolve because of the advantages they give to the survival and reproduction of individuals—that is, by natural selection in Darwin's sense.

Ghiselin has qualities which adapt him for tackling these questions. His philosophical position is sound (that is, I agree with it); he distrusts teleological explanation, despises Baconian induction, and regards holism as an illegitimate attempt to smuggle teleology back into biology. He has a sensitive nose for Panglossianism, which can be defined as the belief that the characteristics of organisms exist to ensure the survival of species and of ecological communities. Although accusations of Panglossianism are usually indignantly denied, such views are widespread among evolutionists, ecologists and ethologists, as Ghiselin makes clear. Equally important, he has the widespread knowledge of natural history which is needed to test evolutionary hypotheses.

Why then does he fail? Ultimately, because he does not think as a geneticist, and because he abjures mathematical language. He rightly remarks that if a mathematical model fails to

Understanding Homosexuality: Its Biological and Psychological Bases. Edited by J. A. Loraine. Pp. 217. (Medical and Technical Publishing: Lancaster, October 1974.) £6.50.

HOMOSEXUAL behaviour is of major interest both scientifically—because of the general problems of the sources of sexual preference, gender identity and gender behaviour—and socially, because of the legal, occupational and other disadvantages suffered by those whose preference is homosexual. Three of the chapters in this book fall under the scientific heading and five under the social heading; the remaining chapter is concerned with venereal disease and homosexuality.

Two of the scientific chapters are reviews, one mainly on males, by Cooper, and one solely on females, by Kenyon. Both are reasonably adequate on the biological sources of homosexual behaviour—Kenyon particularly so—and on parent-child relationships, but neither pays more than passing attention to the potential contribution of the psychology of learning to the acquisition and maintenance of homosexual behaviour. Classical and instrumental paradigms and direct as well as

modelled experiences are likely to be relevant—as they are for all kinds of behaviour. Freund *et al.* report an interesting series of experiments which sought, but did not find, evidence for bisexuality of response. In all three scientific chapters almost all reported studies compared heterosexuals with undifferentiated homosexuals, rather than using the heuristically more useful division into primary (life long) and secondary (non life long) homosexuals.

The remaining chapters are concerned with the legal, theological, and general social responses of the heterosexual majority to the homosexual minority. Grey contributes a particularly thoughtful, sane and eloquent plea for the equality of status of all sexual variations involving consenting partners, both as objects of scientific study and in terms of social esteem. Ramsay and others describe the rapid progress towards equal status in Holland. A final chapter, on the status of the homosexual, by Chew *et al.*, skims rapidly over both social and scientific aspects and includes the provocative assertion that heterosexuality is the "primary cause" of the "dilemma of overpopulation".

Philip Feldman

predict the nature of reality, it is the model which must be changed. But models do make clearer what is being assumed (even if tacit or unconscious assumptions are made, as is often the case, a careful examination of the model will usually reveal them), and they show more certainly whether the conclusions follow from the assumptions. Ghiselin seems to me to fall down on both these points. For example, on page 69 he offers an explanation for the association between diploidy and complex multicellular structure. I simply cannot form a clear picture of the genetic and selective mechanisms being proposed.

On the still more crucial point of the short-term individual advantage of sex, my difficulty is different. I do understand, in a very vague way it is true, what is being proposed. The snag is that I am familiar with several attempts to make these proposals more precise. It turns out that the conclusion—an immediate advantage for sex—seems to follow only if one makes rather extreme and implausible assumptions. Ghiselin would presumably retort: so much the worse for the model. Oddly enough, I agree with him. Plausible models giving an individual advantage to sex may well be possible; Williams and Mitton have made a promising start. But it simply does not do to ignore the difficulty and to appeal to nature as a witness. If we do not have a clearly formulated theory we do not know which facts support it.

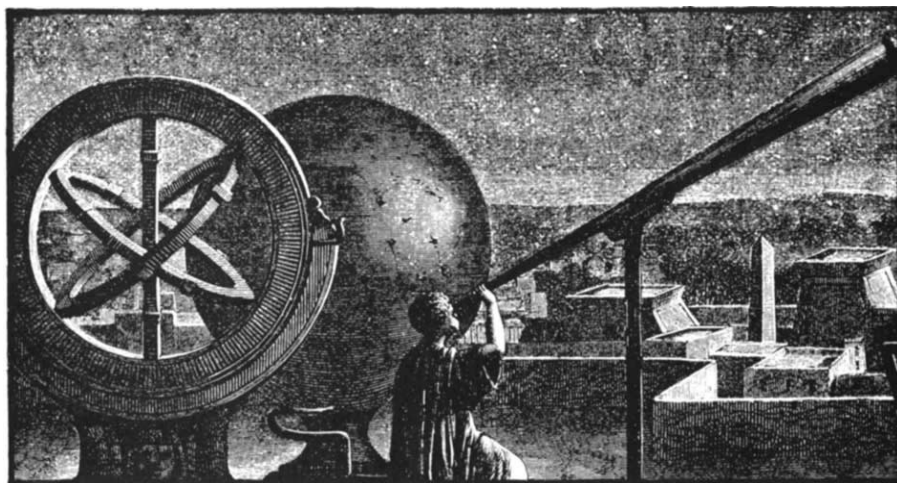
Ghiselin's reluctance to think genetically lets him down in other contexts—for example, in his discussions of the role of kin selection in the evolution of societies, and of Fisher's idea of a positive feedback on female choice in sexual selection (it is uncharacteristic that he does not ascribe that idea to Fisher; one of his virtues is his respect for history). Thus, he seems to regard kin selection as a needless complication, only to be invoked if nothing else will do. I would argue that it is a process which is bound to operate whenever relatives live close to one another, and which therefore must be considered in any discussion of the evolution of societies. I suspect Ghiselin would dismiss this as *a priori*; in fact, I am asserting that if we accept the (empirically tested) theories of genetics, then certain things follow, among them Fisher's and Hamilton's arguments. Of course, the relative importance of these and other processes have to be investigated in particular cases.

There is much in the book that is stimulating and illuminating, particularly in the discussion of sexual selection. But in some ways Ghiselin invites an ungenerous review. He is curiously dismissive of other people's ideas, with the exception of Darwin's. It is true that Panglossianism is rife, but it is not universal. Other evolutionists have been attempting non-teleological explanations of these phenomena. Ghiselin may have more allies than he thinks.

J. Maynard Smith

**The Economy of Nature and the Evolution of Sex.* By Michael T. Ghiselin. Pp. xii+346. (University of California: Berkeley, Los Angeles and London, December 1974.) \$12.05; £7.10.

Covering the Universe



Astrophysics of Gaseous Nebulae. By Donald E. Osterbrock. Pp. xiv+251. (Freeman: San Francisco and Reading, January 1975.) £8.90.

In the Milky Way and in other galaxies the very brightest stars are generally surrounded by masses of bright interstellar gas. These are the H II regions, so called because the hydrogen there is in the form of H^+ . Other atomic species, notably helium, are also largely ionised as well.

All of these ions are continually recombining with the electrons present. If recombination takes place to an excited state then the resultant downward cascade of the recombined atom or ion produces a rich spectrum of emission lines. The optical observation of H II regions relies on these lines; the radio observer can also detect spectral lines arising from transitions between very high levels of the atom concerned. H II regions show up in the radio continuum as well. The emission process here is free-free emission by an electron in the field of an ion.

In principle, the observations contain an enormous wealth of information about the density, temperature, and composition of the gas in H II regions, and its distribution in space. But to tap this wealth requires a considerable amount of physical analysis. Much of the required work has been done by Don Osterbrock and his colleagues; in this book he presents an account of the subject which is so good that it should be unnecessary from now on to consult any earlier work.

The processes by which an equilibrium is attained in a diffuse mass of gas sitting around a bright star which is rich in ultraviolet radiation include radiative transfer and electron collision processes; since many different types of atom are involved the situation becomes quite complicated, but the

author never lets it get out of hand. For good measure he also deals with planetary nebulae, where many of the same phenomena occur on a smaller scale.

But there have been many developments in our understanding of H II regions since the text was completed (about three years ago) and in particular much attention is now being focused on compact H II regions. These are objects whose linear dimensions are smaller. They are often heavily obscured by dust, and are therefore observed mainly at radio and infrared wavelengths. There is a definite association between dense molecular clouds and these compact objects. Very probably they occur in regions where star formation is still active, and the stars involved in them must be among the youngest objects in the sky. Some of these developments are hinted at in the book, but I hope that Professor Osterbrock will be persuaded to write a second, more detailed, volume to deal with these more recent discoveries.

F. D. Kahn

Interstellar Communication: Scientific Perspectives. By Cyril Ponnampertuma and A. G. W. Cameron. Pp. 226. (Houghton Mifflin: Boston, 1974.) £3.50.

ONLY TWO serious possibilities exist for acquiring information about extraterrestrial life. The first is by direct rocket flight within the Solar System. It is possible, though by no means sure, that one of our sister planets may possess a primitive biosphere. The second is by interstellar communication with remote technical communities elsewhere in the Galaxy. With the declining interest in the spaceflight programme, the exciting if somewhat exotic, possibility of radio communica-

tion with extraterrestrial civilisations is achieving mounting respectability and being given serious consideration by astronomers, biochemists, physicists and even social scientists.

In the summer of 1970 a conference took place at NASA's Ames Research Center to bring together acknowledged experts on this nascent subject. The presentations at that conference have now been made available in this book. Enthusiasts will recognise the names of the contributors—Sagan, Drake, Bracewell, Arbib, McCarthy, Aranoff, Oliver and Morrison, as well as those of the editors, Ponnampertuma and Cameron. The book is a well-balanced and eminently readable account of all the varying aspects of this intriguing and daring topic. Although this work constitutes a serious and in-depth study, including technical material of interest to experimentalists, the informal style and interdisciplinary profile of the subject matter will command a wide readership from non-specialists of many disciplines. (It also contains a 39 page bibliography.)

The feasibility of radio communication with extraterrestrial civilisations depends heavily on the proportion of stars in the Galaxy which are likely to support life-bearing planets with technical communities capable of transmitting, receiving and interpreting radio signals. Surprisingly, the most uncertain parameter which determines this proportion turns out to be a social one—the mean lifetime of technical communities. The contributors apparently share a strong opinion that the physical and biological conditions necessary for the formation of life are extremely common throughout the Universe and that given the appropriate type of star, intelligent life of some sort is almost inevitable. Carl Sagan comes up with a formula which predicts that the number of technical communities in the Galaxy is 10% of their mean lifetime in years. This implies the curious result that either the Galaxy is teeming with intelligent life, or, if our civilisation is typical, we are already doomed.

With the optimistic assumption that the technical phase lasts billions of years (there is plenty of time available since the Galaxy was formed) then there may be a few hundred million communicative planets around us.

The formidable problem which then emerges is where to look. Even the optimistic estimates indicate the necessity for a search pattern covering thousands of stars out to a distance of perhaps 1,000 light years—a daunting prospect. Nevertheless, in the early 1960s Frank Drake monitored two nearby stars at 21 cm wavelength in the famous Project Ozma, in the hope of detecting intelligently directed signals. A vastly more ambitious programme is

proposed by Bernard Oliver, arising from a careful study which culminated in Project Cyclops, a summer study at Ames in 1971. This project involves the proposal for the construction of a huge array of 100-m radio dishes spread out over about 100 km², to search a million stars out about 1,000 light years away. This search might take many years to complete, but in view of the cosmic importance of the project, such a long term allocation of resources is deemed to be justified. Oliver devotes part of his paper to the discussion of concrete proposals for hardware and experimental procedures. The system could also be used for transmitting, but in view of the fact that we must be by far the youngest technical community in the Galaxy, it is assumed that the responsibility for this rests on other (extra-terrestrial) shoulders.

Some lively discussion is presented about how two communities who know that they want to contact each other but don't know where to look ought to go about attracting attention. Simple radio beacons are an obvious if rather unsubtle procedure. Dumping a rare

metal into the local star to bewilder distant spectroscopists is another suggestion. Bracewell discusses an intriguing conjecture that probes may be sent around the Galaxy to lie in wait near a hopeful system. If radio signals are received it immediately beams them back as a delayed echo, thereby attracting the attention of an assured audience. Novelties of this sort, though purely speculative, are nevertheless of great general interest and this book is to be thoroughly recommended as a good all-round introduction to the subject.

One final point. Most of the contributors reject the possibility of direct spaceflight between galactic communities because of the vast distances and uncertain destinations involved. It is difficult to fault their reasoning. Anybody who is uneasy about signalling other, possibly bellicose, intelligent beings need fear no invasion. In any case, even Marconi's pioneering signals are now a mere few dozen light years out in space and very probably haven't yet reached any interested ears.

P. C. W. DAVIES

Action at a Distance in Physics and Cosmology. By F. Hoyle and J. V. Narlikar. Pp. x+266. (Freeman: San Francisco and Reading, October 1974.) £7.80.

THE main purpose of this book is to demonstrate that physical facts which are usually explained using field theory can also be explained on the basis of action at a distance between particles.

The book is concerned mainly with electrodynamics (both classical and quantum) and with the masses of particles, which are treated as arising from interparticle action in accordance with Mach's principle. No attempt is made to give gravitation an action at a distance form and the identification of the gravitational field with the metric is retained.

The direct interaction between particles is symmetrical in time, but to this must be added the effects of the 'response' of the rest of the Universe. To be consistent one must obtain a cosmological model in which this response can lead to the usual purely retarded interaction. In the case of the electromagnetic interaction this condition requires that the Universe be a perfect absorber in the future. Of the usual models those satisfying this condition are static universes, the closed Friedmann model, and the steady state model. The static models can certainly be rejected on observational grounds, and most people, including apparently the authors of this book, would now also reject the steady state model. In spite of the statement to the contrary on page 174 (which is based on the misleading principle of taking the Einstein-de-Sitter model as typical of the Friedmann models) the closed Friedmann model also satisfies the response condition for the mass field. The authors, however, reject this model on the grounds that although retarded potentials are consistent, so are advanced potentials, so that the cosmological model does not define the direction of time. Their conclusion is thus that the Friedmann models must be rejected altogether in favour of some more complex model, not specified in detail. If, however, the choice were between abandoning the Friedmann models and deriving the direction of time from some source other than cosmology (such as thermodynamics) then I think most physicists would choose the latter.

I have concentrated on the book's discussion of cosmology, since this seems to be crucial to the whole theory. The book is, however, by no means written solely for cosmologists and it contains much to interest a wider audience. I doubt, though, that it will convert many to the action-at-a-distance viewpoint. G. J. SUGGETT

Everyman's Astronomy. Edited by R. H. Stoy. Pp. 493+48 plates. (Dent: London, January 1975.) £4.50.

ANYONE who has followed the development of astronomy over the past few exciting years will realise that this otherwise excellent book is about four years out of date. Black holes are not even mentioned in the index, X-ray sources receive scant attention, and even the discussion of pulsars is most notable for its omissions. Perhaps most seriously of all, given the readership for which the book is clearly intended, the recent probes to Mars (Mariner 9), Venus and Mercury (Mariner 10) and Jupiter (Pioneer 10 and 11) all produced their dramatic new data after *Everyman's Astronomy* was ready for the press.

There is, of course, a reason for this unfortunate gap. The volume, intended to replace the successful *Astronomy for Everyman*, was conceived in 1960 when several chapters were commissioned, but was later abandoned until 1969 when Dr R. H. Stoy was called upon to complete the project. As he says in a preface, "the epoch of the book as a whole is approximately 1970"; but further delay arose because of the need for extensive editing to ensure homogeneity and to reduce any overlap in contributions gathered over the best part of a decade.

With hindsight, there is no doubt that the publishers would have done better to wait for another few years

and produce the definitive book of its kind for the late 1970s. The present round of exploration of the Solar System is now complete, and there is likely to be a lull until Mars landers, and the passage of Pioneer 11 past Saturn, revive interest in the late 1970s. In other areas of astronomy, the entire electromagnetic spectrum is now open to view from satellites, including γ rays, X rays and infrared radiation, so that again we are standing at a watershed in the development of the science.

But the failure of the book to have caught just the right tide in the affairs of astronomy only assumes importance because what is covered here is covered so well. With nine first rate contributors, including Professor V. C. Reddish, Professor Z. Kopal and Professor David Evans, it would have been surprising had the text not been of a high standard. The illustrations—photographs and line drawings—complement the text well, and with nearly 500 pages of lightweight paper packed into the volume of a pocketbook the only notable omissions are those caused by the publication delays.

Not least among the assets of the book is its price, which puts it well within the reach of all serious amateur astronomers. They would be well advised to acquire this book. But even though it is aimed at such readers, not a few professionals (and certainly all astronomy students) are likely to find it valuable for its compact presentation of a wealth of basic data.

John Gribbin

Semiconductors

Amorphous and Liquid Semiconductors. Edited by J. Tauc. Pp. ix+441. (Plenum: London and New York, 1974.) \$33.60.

INTEREST in the field of amorphous semiconductors has increased rapidly during the last 10 years because of the obvious commercial success of the Xerox process and the possible commercial success of the amorphous switching device. Before that, technical interest in the amorphous state was sustained by those interested in photoconductors as potential light sensors and in glass technology; and fundamental studies derived largely from interest in the liquid state and in the development of general methods for defining order and disorder in solids.

The graph of interest against time has probably now flattened off and major discoveries are appearing more slowly; meanwhile, several reviews of the field have appeared in book form. It is a good time to ask if these books tell us whether we have got anywhere in understanding amorphous semiconductors, and whether it is worth sustaining any interest. The questions come to mind because the first clear message from all of the works is that the fundamental problems of under-

standing amorphous solids are very difficult, and the experimental problems, such as control in preparation and the significance of measurements, are considerable. Will the strenuous efforts which are still required to solve the problems repay us to a similar extent as, say, an equal amount of work on new crystalline semiconductors?

The collection of chapters edited by Dr Tauc certainly has the depth to give the reader a chance to address the question: the extensive and thoughtful reviews are presented by some of the most respected workers in the field. To this depth the editor has added a suitable breadth by selecting authors who have a suitable diversity of viewpoint. Some freshness is added by the inclusion of authors who have not yet received very wide exposure.

Bagley, a less well known author, draws a distinction between glasses and other amorphous materials, emphasising that the structure obtained from the cooling of a liquid will be "structurally and thermodynamically related to that liquid" but that similarities between those glasses and "other amorphous solids" may be tenuous. Such a caveat is a useful corrective to carry into the other chapters, which seek mainly to explain the electrical and optical properties of that class of 'other amorphous solids'.

Of the chapters which review large volumes of data, Tauc's and Fritzsche's are excellently condensed, although Tauc sometimes relapses into single-sentence listing without further observation and, in the end, shirks a conclusion. Fritzsche, by contrast, ends his chapter on electronic properties with a precise and frank summary of the gulf which still exists between experiment and theory in the field of electrical transport. He stresses that many amorphous semiconductor films contain heterogeneous voids and that this may invalidate many of the theories based on measurements of conductivity, elastoresistance and thermopower.

In his second chapter, Fritzsche gives a summary of the chemical requirements for the components of a switching or memory alloy. In such paragraphs, one feels that one is getting the distillation of a huge amount of research in a few sentences and that the book is thereby achieving its intention—that of a mature review.

Girgovici has managed similarly to condense knowledge in a lively review of the mode in which atoms pack and bond in known amorphous materials. Economou, Cohen and others give a highly condensed summary of the necessary band and percolation theory.

The book represents a useful reference volume and is a very good starting point for those who wish to understand the field.

A. G. Holmes-Siedle

Affinity chromatography

Affinity Chromatography. By C. R. Lowe and P. D. G. Dean. Pp.xi+272. (Wiley-Interscience: London and New York, September 1974.) £6.25.

THIS book would be a valuable asset to any research group working in fields such as enzymology and protein chemistry in which an awareness of the principles and potentialities of affinity chromatography must now be considered obligatory. The original literature on affinity chromatography is now considerable and an introductory text was badly needed. Both the length and the price of this book have been well judged.

The reader should not be put off by the first part of the introduction in Chapter I as I almost was. It consists of three pages on protein structure, which are entirely superfluous for anyone who is at all familiar with proteins, and which certainly would not help anyone who was not. The flavour of those three pages is unfortunate: for example, the common acidic and basic groups of amino acids are wrongly assigned as in the worst undergraduate texts, peptide bond formation is stated to "occur" by elimination of a water molecule, of all things, and that is illustrated by a "lasso" in Fig. I.1. The structure of the indole ring of tryptophan (Table I.1) is wrong and the hydrogen atom of the imidazole N-H bond is missing, as indeed it is throughout the book.

Happily, after that the presentation improves dramatically. Most sections provide a short but helpful discussion of theoretical aspects followed by interesting examples well illustrated by elution diagrams and by structural formulae where appropriate. This is particularly helpful in a book that deals with many different types of molecules and which is aimed at a wide readership that must include people not normally used to thinking in molecular terms. Although many of the theoretical sections are, of necessity, brief they do provide very useful starting points for further reading. All sections of the book are well supported by literature references up to mid 1973. Following an introductory chapter, there are four main chapters. These deal with the principles of affinity chromatography; group specific adsorbents; various applications of affinity chromatography, including hydrophobic chromatography; and the organic chemistry of materials used in affinity chromatography, particularly the reactions involved in the preparation of various affinity adsorbents.

K. Brocklehurst

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To follow a flow

Flow Visualisation. By Wolfgang Merzkirch. Pp. viii+250. (Academic: New York and London, October 1974.) \$26.00; £12.50.

THE need for reliable and accurate methods for rendering fluid flows visible arises in a wide variety of situations in aerodynamics, chemical engineering and fundamental experimental studies in fluid dynamics. Many different techniques have been developed over the years and major advances in the subject have occurred quite recently. For that reason alone there has been a need for a book which collects together all these techniques and brings the subject up to date. This book fills the need adequately. In an exhaustive survey the author describes, with the appropriate theoretical background, the methods from many fields, and he identifies the conditions and parameter ranges under which each method is applicable.

Merzkirch divides the subject into three classes: over half of the book is given over to the classification which is concerned with the various optical arrangements available for obtaining both qualitative and quantitative information from fluid flows. This classification, which is headed rather loosely "Optical methods for compressible flows" (even though, in fact, several of these methods have been successfully used in incompressible flow situations—schlieren systems in salt stratified fluids, for example) describes in detail techniques such as shadowgraph and schlieren arrangements which are based on the change in refractive index of a fluid as a function of density. There are particularly interesting contributions on holographic flow visualisation and the laser-Doppler anemometer, though it is doubtful whether its inclusion in this book is strictly justified.

The second division comprises techniques which rely on the addition to the flow of foreign material such as dye. Attention is given to the basic criterion that the flow should not be disturbed by either the mode of injection or by the material itself, and a series of empirical rules, ranges of validity and necessary corrections are given for the various methods. This section, which is descriptive in character, contains useful information on the more recent innovations such as temperature-sensitive paints, liquid crystals and flash photolysis, as well as coverage of the more traditional methods.

The remainder of the book is taken up with a discussion of special problems and techniques and a third classification

headed "Flow field marking by heat and energy addition". The methods described in the book cover a very wide range but are confined essentially to laboratory scale flows. For completeness it could have included some of the special techniques used in larger scale field observations (estuary flows or oceanographic phenomena, for example).

Nevertheless, the book is well written and contains a large number of excellent photographic illustrations, and it will be very useful to experimentalists working in many branches of fluid dynamics. **P. A. Davies**

Life time of an organism

Living Clocks in the Animal World. (A Monograph in American Lectures in Environmental Studies.) By Miriam F. Bennett. Pp. xiii+221. (Charles C. Thoms: Springfield, June 1974.) \$11.75.

CIRCADIAN and other biological rhythms are attracting increasing attention every year. Strictly speaking, however, we still do not know how their extraordinary 'clock' properties are achieved. The majority of investigators, myself included, consider that the timing of circadian rhythms is derived from the chemistry and physics of the cell or organism. At least one laboratory, however, led by Professor F. A. Brown, believes that the timing is provided by "subtle geophysical factors" associated with the solar day, and, incidentally, not excluded in most experiments.

The author of this small volume has been one of Brown's associates, and when I opened the book I expected to find that she pressed the second of these views. She presents both cases quite fairly, however, and interprets many of the phenomena in endogenous terms. She points out that the 'exogenous school' does recognise that organisms are "equipped with cellular clocks", and that the 'endogenous school' recognises that environmental variables, particularly changes in light intensity and temperature, can modulate overt rhythms in a manner which is distinct from their rôles as entraining agents. She considers, perhaps correctly, that a "spectrum" of clocks from "purely" endogenous to "purely" exogenous exists in the animal world.

Her material is, however, limited to those groups which she knows best, and she ignores almost totally the work done on birds, mammals and insects, which has provided most of our knowledge on circadian systems. For this reason, I would not recommend it to any of my students. **D. S. Saunders**

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Biochemistry

The Development of Biochemical Concepts from Ancient to Modern Times. By Henry M. Leicester. Pp. 286. (Harvard University Press: Cambridge, Massachusetts, 1974.) \$15.00.

THIS is a book which was very much needed, and happily Professor Leicester has made a real success of it. Apart from a group of lectures at the Cambridge Biochemical Institute, recently published, the only thorough treatment of the history of biochemistry was the book by F. Lieben, *Geschichte der physiologische Chemie*, published 40 years ago. Lieben's book has been reprinted in recent times and will always have its value, but it is not satisfying nowadays for a number of reasons. For example, he disposed of the entire period down to the end of the 17th century in only 20 pages out of a total of 700; then he divided the main body of his text into physiological topics, which were followed by an account of discoveries in the present century grouped under proteins, carbohydrates and fats. The attitudes of the early thirties are very dominant, and as a whole the work lacks historical perspective.

This state of affairs is now vastly improved upon in Leicester's book. Eighty pages are devoted to the period from antiquity to the end of the Middle Ages, 45 to the 17th century and the time of iatrochemistry, and the remaining 105 to biochemistry, as the child of modern chemistry and modern biology. The book is well informed in classical and mediaeval history, yet comes down as far as the discoveries about the genetic code made by Watson and Crick in 1953, the latest references being to work in 1971.

The discussion of the ancient Western world draws from the pre-Socratic philosophers and the Aristotelians, with a paragraph or two on Alexandrian 'alchemy'. Far beyond the ken of Lieben, there is a good chapter on biochemical ideas in ancient China and India, now rightly regarded as necessary because of their great influence on Arabic culture, without a consideration of which the history of any science in post-mediaeval Europe can never properly be understood. Leicester has a clear appreciation of their macrobiotic drive, as opposed to the more metallurgical aurifictive and aurifactive approach of the Hellenistic papyri and the Corpus writers, respectively. The Arabic chapter is also well done, though perhaps the ideas in the Jabirian Corpus (p.56) are passed over a little too quickly. On the other hand, it is a good idea to emphasise the dawning of the ideal of the necessity of quantification, which occurred in the late Middle Ages with men such as Nicholas of Cusa.

It has been said that most of the early history of biochemistry can be followed by tracing all the peregrinations of certain fundamental ideas—such as *pneuma*, element, humour, *krasis*, quintessence, elixir, conjunction and ferment. Only four of these are actually in Leicester's index, but two more of them are fairly readily found within the text, and the reader can easily add the references to the index in his own copy. Although the Paracelsian chapter is so good, with its explanations of 'chaos', 'tartar', 'iliaster', and 'arcanum', one would like to have seen the concept of 'elixir' followed through somewhere; and again the ancient idea of the *conjunctio oppositorum* lying at the basis of all affinity theory, could also have been brought in with advantage. The great influence of the first distillers of strong alcohol is relevant here, since *aqua ardens* seemed indeed to be an instance of the "marriage of fire and water".

As an offering towards a second edition, I would express regret at the attribution of the discovery of gluten only to Grimaldi in 1665 (p.118), since *mien chin* had been systematically prepared on a nation-wide, if not indeed an industrial, scale in China for at least a millennium before. Indeed, the discovery may well go back to the second century BC if the traditional ascription to Liu An and his circle turns out to be justified. I would also like to see a rewording of the statement on p.49 that a characteristic feature of Chinese medicine was its reliance on herbal medicines. This is particularly misleading because the Chinese in their pharmaceutical natural histories from the second century BC onwards never shared the Galenic abhorrence of non-herbal remedies. From the beginning their physicians made much use of mineral drugs, and animal products were 'officinal' from the beginning. Thus, no Society of Chymical Physitians was ever necessary to awaken the Chinese from their Galenic slumbers; they had always been awake to the three kingdoms.

A great attack on Galenic orthodoxy took place when the specific virtues and specificity of drugs, as opposed to the unspecific adjustment of 'peccant humours', were recognised in the 16th and 17th centuries (pp. 86, 98). It might have been good to instance at this point the remarkable career of the Cambridge 'quack' physician, Talbot, who administered quinine widely and with great success in defiance of the reigning doctrine. Still later we get involved in the vitalism-mechanism polemics of the 19th and 20th centuries. Perhaps it is not quite enough to end the discussion (p.159) by saying that "with the accumulating mass of chemical and physiological information

vitalism gradually disappeared from biological thought". Would not a page or two of the current debates, centering round 'reductionism', have been in place here? They contain much of great interest for the philosophy of science, and the general position of organicism is so significant in the light of more than 2,000 years of past history, that this might well have been expanded. Lastly, I should like to offer to the author for a footnote in his second edition, the tradition that the invention of the term 'hormone' (p.228) actually occurred in the Hall of Caius College at Cambridge, when Bayliss and Starling were dining at High Table and had a conversation about a word they were looking for with the Greek and Latin scholars who were of the company.

Professor Leicester's book is well provided with exact references, the absence of which was one of the worst features of Lieben's book. The new book can be recommended without reservations to students and research workers alike, but of course it is not the last word on the subject. One can see a place which could be filled by something at one and the same time more philosophical and more industrially-oriented than either Lieben or Leicester; meanwhile let us be grateful for this book.

Joseph Needham

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Developing form

On Development: The Biology of Form.
By J. T. Bonner. Pp. 282. (Harvard University Press, Cambridge, 1974.) \$10.00.

Do not expect to find here a fashionable account of developmental biology in terms of molecular mechanisms controlling transcription and translation, or specificity of cell surfaces, or automata theory. For this book attempts to deal with developmental biology in a very broad manner, by considering not only mechanisms in a general way but by considering also the problems of evolution and population biology.

Bonner attempts to do this by emphasising the importance of life cycles, which he believes throws new light on basic problems. He views, for example, biological reproduction in terms of reproduction of the life cycle and this theme is expanded in the first part of the book to discuss size increase in evolution, senescence, variation, the origin of life multicellularity. An essential feature of a life cycle is not only to reproduce a new cycle, but to get rid of the old one, and he thus argues for programmed senescence. (No mention is made, however, of Hayflick's studies on the limited life span of somatic cells.) Some attention is given to looking at life cycles as having points of maximum and minimum size, and size increase during evolution is discussed in some detail from the point of view of selective advantage. There is also a quite long digression on the origin of social organisms. His aim is to say something about the larger significance of development, such as why development occurs, how it evolved, and its relationship to all other aspects of life. Interesting though this biology may be, I have not found viewing it in terms of life cycles very helpful, nor do I consider it to be an advance over the distinction between genotype and phenotype. By looking at the selective advantage of the phenotype he misses the really interesting questions on the relationship between evolution and development. What we want to know is what constraints and integrations development provides when the genotype changes. How, for example, do new cell types arise? What is the selective advantage as they are evolving? One can ask the same question of the evolution of structural innovations such as the wing. Again, what sort of changes in genotype are required in the alteration in the pattern of muscular activity. Must one alter genes controlling both nerve and muscle, or will developmental mechanisms provide the required integration? Could one by appropriate selection evolve a bat-like wing for man? Dobzhansky has said no, and thus man has no hope of

becoming an angel, but I do not believe current theory permits a decision. None of these problems is even recognised in this book.

The second part of the book is concerned with the mechanisms of development and covers topics such as synthesis of substances, timing, localisation of substances and control of pattern, and is rather disappointing. Part of the difficulty is that Bonner seems much more at home with protozoa, fungi, slime moulds and plants. This may account for the fact that most of the examples are drawn from such fields and not only are they less familiar, but in general, much less is known about them. This also accounts for the rather cursory treatment of topics such as microtubules and microfilaments and cell movement in general. One could argue that for animal development this was an area of major importance in which rapid advances are being made. Other areas such as clonal analysis, which are being applied with remarkable success to insect development are not even mentioned.

The book is characterised in places by a regrettable lack of rigour. For example, the brief analogy drawn between the brain and the structure of the genome is very superficial, in that it would be as true of a juke box. Again, a principle of convergence is invoked to suggest that particular biological functions in development can be fulfilled in a variety of ways; but no attention is given to a principle of conservation which would suggest that once embryos have found a good way of doing things they will continue to use it. In fact, one of the major failures of the book is to bring out common features and general principles in development. Yet again, the author suggests that it is a distinct selective advantage for the grouping of developmental events into superunits of gene action, but very little evidence is available to substantiate such a view: the interconnectedness of genes remains a major problem and is not seriously analysed here. Later, he suggests that there are not enough genes to account for all individual neurones, completely ignoring the tremendous combinatorial possibilities of gene networks which could easily and uniquely characterise each neurone with a relatively small number of genes.

In spite of these criticisms, the author draws on a wide range of biological examples and his ideas are often provocative and original. By raising problems ranging from the development of the social behaviour of ants to the form of mushrooms he presents to developmental biologists problems and ideas that rarely cross their minds.

Lewis Wolpert

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Seals, monkeys, apes and rabbit



A COMPREHENSIVE work on British seals is long overdue and one by an authority as eminent as the late Emeritus Professor H. R. Hewer is particularly welcome*. Although seals are among the largest of British mammals, before the last war comparatively little was known about them. Since 1947, however, an increasing amount of research has been undertaken and the published results form the backbone of this book. Hewer started his own research in 1951 and during the following years he visited all the major British colonies, with the result that this is no 'scissors and paste pot' work; it is something that not only bears the stamp of genuine knowledge, but also includes many personal observations.

The first chapter is devoted to a brief account of the Pinnipedia and their evolution into three dissimilar families; it also describes their anatomical features and shows how these have become adapted to a marine environment. The main part of the book concentrates on the life history of the two seals that breed in Britain, namely the grey seal *Halichoerus grypus* and the common (or harbor) seal *Phoca vitulina*, and it is a measure of the present state of knowledge of these animals that, whereas the grey seal warrants nine chapters, information about the common seal can be contained in two.

The remaining seals on the British list are also discussed and short accounts are given of the walrus and the four species of phocids that appear

as vagrants. Mention is made, too, of the occasional 'escaped' Californian sea lion.

A final chapter which deals with the vexed question of seal conservation and management, should be compulsory reading for all those who think of seals only as appealing white-coated babies. Five appendices and a comprehensive bibliography complete the book.

There are numerous illustrations and maps, some of the most useful being Hewer's own line drawings. The photographs vary in quality and, although some of the 'chalk and soot' can be excused on the ground of enlargement from colour film, at least two have already been reproduced much more successfully elsewhere.

My most serious criticism is levelled at Appendix D and, as the source of the basic data, I think it unfortunate that Hewer did not consider other possibilities before publishing his theories. There are other minor errors which someone with an intimate knowledge of a particular colony is bound to discover, but, these apart, *British Seals* is a book that can be recommended to anyone, whether amateur or professional, who is interested in these controversial mammals.

Grace Hickling

ONE of our most pressing problems, so we are continually told, is our aggressiveness. Let it rip and you may end up with a lifer, bottle it in and you get ulcers. The answer, they say, may lie with the monkeys. Although competition for food or attractive females is common in most natural groups and the majority of species possess the

equipment for easy murder, killing and serious wounding are rare. Holloway's book† provides no panaceas and includes little material of direct relevance to human aggression. It does, however, draw together current knowledge of several aspects of aggression in primates.

Seven chapters describe the patterns, distributions and contexts of aggressive interactions in different primate taxa: tree shrews, nocturnal prosimians, diurnal prosimians, ceboids, colobines, cercopithecoids and pongids. A further two describe the reactions of rhesus monkey groups to the introduction of additional animals. Two important, if obvious, themes recur throughout these chapters. First, aggression is the means by which individuals maintain access to food, females and territories. Second, the frequency, context and distribution of aggressive interactions differ between species, between populations of the same species and (though it is hardly emphasised here) between individuals; in most natural populations of primates, aggression is both flexible and adaptive. Both points need to be borne in mind when we consider our own predicament.

A general weakness of this group of chapters is that too much space is devoted to the description of trivial interspecific differences in aggressive behaviour and too little to the generalisations and theoretical implications arising from the surveys. The excellent chapter by Nagel and Kummer on cercopithecoids (Old World monkeys) is a marked exception and emphasises how much more is known about the behaviour of this group compared with the others.

Research on the effects of androgens, brain stimulation and neural lesions on aggressive behaviour is reviewed, and interspecific differences are related to variation in neuroanatomy. These reviews show that behavioural aspects of research in this field lag some way behind naturalistic studies in sophistication (though the automatic recording systems described by Maurus offer the possibility of a speedy reversal of the situation). The effects of physiological manipulations on aggressive responses given in different contexts or to different classes of individuals are rarely distinguished, and the numbers of animals tested are usually extremely small. This is an important weakness as there is evidence that the physiological controls of aggression are both complex and variable; that is demonstrated by the useful review of the effects of androgens by Rose *et al.*

†*Primate Aggression, Territoriality and Xenophobia*. Edited by Ralph L. Holloway. Pp. xiv+513. (Academic: New York and London, 1974.) \$29.50.

**British Seals*. By H. R. Hewer. Pp. 256+24 plates. (Collins: London, November 1974.) £3.50.

Two final chapters by J. P. Scott and C. R. Carpenter review the field on a wider basis. Both are more concerned with easy generalisations about primate aggression—males are more aggressive than females, aggression occurs in competitive situations and is influenced by crowding or changes in group membership—than with important problems. That is a pity, because several theoretical problems occur throughout the book and require detailed consideration. Just what is aggressive behaviour? (It is defined in at least 10 different ways in as many chapters). Is predatory behaviour controlled by the same mechanisms or not? Should aggression include threats and displays or just physical attacks? What is the adaptive significance of differences in aggressiveness? If aggression is the means by which individuals maintain access to limited resources, why do they not kill subordinates and have done with it? When discussing the functions of aggressive behaviour, too many of the contributors treat aggression as a syndrome, suggesting advantages which invoke group selection. In contrast, there is too little discussion of the adaptive significance of differences in aggressiveness between individuals or between contexts.

Finally, how much do primate studies really tell us about humans? Clearly, research on the control and development of aggressive behaviour in primates suggests possibilities which can be investigated in man afterwards, but it is not obvious that broad, comparative studies help much. Perhaps the proper way to study mankind is not through monkeys after all since man's environment differs so widely from those of other primates. Despite these limitations, zoologists and comparative psychologists should be grateful to Holloway and his contributors for providing reviews which cover a wide and disparate literature.

T. H. Clutton-Brock

Primates: Comparative Anatomy and Taxonomy. Vol. 7: *Cynopithecinae*. By W. C. Osman Hill. Pp. xxxi+934+48 plates. (Edinburgh University Press: Edinburgh, April 1974.) £25.00.

THESE days, when half the new publications in science are either proceedings of symposia or multi-authored surveys of the latest scientific wonder, a scholarly monograph written by a single author is as welcome as the sound of the cuckoo in April. Volume 7 of Dr Osman Hill's encyclopaedic series on the primates is a worthy companion to the previous seven volumes (including Volume 8 which was published out of sequence).

It is not easy to criticise a work of such length and distinction without

giving the impression of cavilling, but where matters of nomenclature and taxonomy are at stake even apparently trivial issues require comment. Two main criticisms spring to mind; there is a tendency to include everything rather than to select, and to cling to the past rather than to come up to date.

To illustrate the first point: two range maps of the same two species are included, showing radically different distributions (Maps 11 and 12, *Macaca arctoides* and *M. thibetana*). To say the least, that undermines the confidence of the reader.

The second point—clinging to the past—is instanced by the use of the name *Macaca irus* for the crab-eating macaque, ostensibly for the sake of uniformity with previous volumes. Since the publication of the *International Code of Zoological Nomenclature* in 1961, the zoological nomenclature game (once a highly esoteric pursuit) has become one which all can play. Anybody with access to a museum library can see for themselves that under Article 11g(ii) the name *irus* was not properly proposed by Cuvier in 1818, and will therefore reject it. The retention of an unavailable name merely tends to boost its status and prolong its use, thus perpetuating nomenclatorial confusion.

Turning from nomenclature to taxonomy, the same backward looking trend can be seen in Dr Osman Hill's handling of the *Macaca/Cynopithecus* controversy. In 1969 Fooden showed convincingly that the seven species (or subspecies) of Celebes macaques are monophyletic, probably an offshoot of Bornean *Macaca nemestrina* stock by way of the Makassar Strait. The species at the centre of dispersal, *M. tonkeana*, seem to be the least differentiated, and the terminal species in the north (*M. nigra*) and in the south-east (*M. brunescens*) are, as might be expected, the most highly differentiated, and are linked to *M. tonkeana* by intermediate populations. Mayr, whose ideas on systematics and the origin of species have been widely adopted, would undoubtedly interpret these forms as congeneric. To quote from his book *Systematics and the Origin of Species from the Viewpoint of a Zoologist* (Dover: New York), "That they are nothing but subspecies, or at best allopatric species, is particularly evident in cases in which the widely diverging species are the extreme ends of a long chain of intermediate subspecies."

Nevertheless, Hill still feels it necessary to give taxonomic expression to the extreme differentiation of the northern Celebes 'Black Apes' (*Cynopithecus*) from the Moor Macaques (*Macaca maurus*), following Laurie and Hill (*List of land mammals of New Guinea, Celebes and adjacent islands, 1758–*

1952. Trustees of the British Museum, Natural History, London). But contrary to Laurie and Hill, the watershed between the two genera has been shifted further south so that *M. tonkeana* (considered by Laurie and Hill as a synonym of *Macaca maurus*) is now arbitrarily assigned to the genus *Cynopithecus*. Arbitrarily? I am afraid so, because no convincing data are given to back up this decision, without which it can carry no weight. It merely serves further to embroil the reader in the confused taxonomy of the group.

The present volume will undoubtedly be welcomed, particularly by applied scientists for whom the macaque monkey is still the centre of the experimental universe. The information and sources that Dr Osman Hill supplies are invaluable to all research workers who will continue to regard his series as the most authoritative and definitive available. One will not see its like again.

John Napier

The Biology of the Laboratory Rabbit. Edited by Steven H. Weisbroth, Ronald E. Flatt and Alan L. Kraus. Pp. xi+496. (Academic: New York and London, 1974.) £23.75.

THIS is the first comprehensive text devoted solely to the laboratory rabbit, and it will prove to be of value to a wide variety of scientists concerned with the use of this species as an experimental animal, or who are engaged in the practice of laboratory animal medicine and husbandry.

There are 25 contributors (all from the USA) who provide 18 chapters. The text is supported by numerous illustrations and tables, together with a comprehensive list of references at the end of each chapter.

Chapters 1–3 consider basic aspects such as genetics, husbandry, anatomy, physiology and biochemistry. Additional genetic information is given in chapters 7 and 15, which are devoted to serological genetics and inherited diseases and variations.

More than half the book (11 chapters) is devoted to disease, parasitic infections and pathology, and successfully amalgamates much of the literature scattered throughout scientific proceedings and journals.

Chapter 4 is concerned with biotechnology and details many basic techniques and procedures.

Chapters 5 and 6 contain comprehensive reviews on the rabbit foetus in experimental teratology and on arterio-sclerosis research, and chapter 8 provides a sound introduction to gnotobiology.

Any attempt to consolidate information economically will leave gaps, and this book is no exception. Nevertheless, it can be thoroughly recommended for study and reference. **John Bleby**

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Everything caudal to the eyes

Handbook of Sensory Physiology Vol. VII/3: Central Processing of Visual Information. Parts A and B. Editorial Board: H. Autrum, R. Jung, W. R. Loewenstein, D. M. Mackay, and H. L. Teuber. (Springer: Berlin and New York, 1973.) Part A: pp. xi+775; part B: pp. viii+738; DM 248 each.

Nor one book but seventeen (at the latest estimate), and each one so weighty that a single hand can hardly lift it. That is the *Handbook of Sensory Physiology*. It will probably exceed 13,000 pages with more than 4,000 illustrations and is undoubtedly intended to be a definitive statement of the progress of research on sensory systems. The list of authors is massively impressive, the production lavish, and the coverage catholic almost to the point of being indiscriminate.

Volume VII is devoted to vision and I am particularly concerned with Volume VII, Part 3, two large tomes which tackle the *Central Processing of Visual Information*. It is edited by Richard Jung, the father of the Freiburg school of neurophysiology, where work on the visual cortex began 25 years ago with the explicit motive of seeking explanations for perceptual phenomena in physiological terms. That theme is well represented in these books, which cover everything in the visual system caudal to the eyes.

Professor Jung opens Volume VII, Part 3, Part A, Section 1, Chapter 1 (believe it or not) with an encyclopaedic account of the successes of his own approach to the visual system, particularly concerning the role of so-called 'B' (brightness) and 'D' (darkness) neurones in determining the perception of brightness and contrast. This chapter alone is about 150 pages long and together with two other chapters of 100 pages or more (by Grüsser and Grüsser-Cornehls on movement perception, and van der Grind *et al.* on temporal transfer properties) it dominates the first book.

In Part B there are five especially valuable comprehensive chapters: Sprague *et al.* give a much needed, up-to-date account of the functions of visual projections to the tectum; Freund covers the physiology of the lateral geniculate nucleus; Brooks and Jung do the same for the visual cortex; and Szentágothai reviews the structure and ultrastructure of both the geniculate body and the cortex in two chapters that are as pleasing to the eye as they are to the rest of the nervous system caudal to the eyes.

It is the major reviews that give this work its historical importance, of

course, but I enjoyed the books more for some of the shorter, more personal chapters on specialised topics: MacKay on the stability of the visual world, Levick on spontaneous and maintained neuronal activity, Gross on the inferotemporal cortex and Brindley on stimulation of the human visual cortex.

All these and many other fine chapters make the books indispensable for any library of physiology. Not that the whole venture is without fault. In general, the books suffer dreadfully from the lack of overall organisation that so often mars multi-author works. In fact, the degree of overlap and even straightforward contradiction between chapters, and the whimsically random systems of referencing make the books frustrating to read. The few chapters on visual processing in lower animals, although excellent in themselves, seem an incongruous sop to comparative physiology. Where is the essential review on the analysis of visual information (not just colour vision) in insects, spiders, octopus, crayfish and so on?

Finally, I am disturbed at the price: £45 for 800 pages. I believe that books of this sort ought not to cost so much; the libraries that will have to take the whole series are going to have to pay a vast sum. It raises in my mind doubts about the role of purely commercial enterprises in the essential business of disseminating scientific results.

Colin Blakemore

Into the cell

The Cell Nucleus. Edited by Harris Busch. Vol. 1, pp. xxiii+667; vol. 2, pp. xxiii+564. (Academic: New York and London, July 1974.) \$45.00; £21.60 each.

DR BUSCH has courageously attempted to collect together all the important knowledge about the eukaryotic nucleus and it has required 59 authors, many of whom have made fundamental contributions to their subject, to write the 39 articles. It is interesting to draw comparison with a similar major work, the six volumes on *The Cell* (Academic: London, 1959-64) which were almost exclusively devoted to the cytoplasm; and with a slim volume, *The Cell Nucleus* (Butterworth: London, 1960). Our knowledge then was quite primitive, but at about that time two fundamental observations were made which have led to the development of important new techniques and thus to many of the discoveries now described.

First, Marmur and others showed that it was possible to separate the two strands of the DNA molecule and then to reassociate them, a process depending on pairing between complementary sequences of the nucleotide bases. This

technique of reassociation, or hybridisation as it is called when the DNA chains are from different organisms, or involves an RNA chain, has led to a new understanding of the nature of the base sequences, their repetition and their location, in the very long DNA molecules thought to comprise each eukaryotic chromosome. In Busch's volume seven articles refer to various aspects of this problem and Strauss makes useful comparisons with the genomes of bacteria and viruses. The most thoroughly understood sequence is that coding for ribosomal RNA (Choi, Nazar and Busch), but the article on the fine structure of the site of its transcription, the nucleolus, may leave readers in a confused state because of the lack of a suitable schematic diagram and because of the variation in the terminology used for the different zones.

Second, in 1960, Barski discovered the spontaneous hybridisation of different cell types. Cell fusion has been called "the new gift to biology" and the reasons will become clear from Sidebottom's excellent article on heterokaryons. Cell fusion is the analogue of gamete fusion and Kucherlapi, Creagen and Ruddle describe its use in genetic analysis of somatic cells. The genetic content of the nucleus can also be studied by implantation into eggs and Gurdon summarises his elegant experiments which show that cell differentiation is not accompanied by loss of, or permanent alteration of, genes.

Five articles, including a lucid, basic introduction by Arrighi, provide a comprehensive survey of mammalian metaphase chromosomes and their recently discovered banding patterns. There are, however, numerous texts on these topics and the article showing clinical pictures of the effects of chromosome imbalance could have been omitted to help reduce the high cost of these volumes. Substantial articles deal with heterogeneous and other nuclear RNAs, nuclear proteins and DNA polymerases, and DNA-dependent RNA polymerases. Franke and Scheer, and Kasper, have contributed extensive and detailed articles on the nuclear envelope and their enthusiasm is evident from the number of recent results added in proof. There are also excellent chapters by Edström and by Hennig on polytene chromosomes. An aspect of chromosome structure, other than the base sequence, is the way in which the DNA molecule is folded up in association with proteins, and Solari skilfully steers us through the complexities, but unfortunately the book is already too out of date to include exciting new results on ν -particles, nuclease-protected fragments and so on.

The Cell Nucleus contains most

of the relevant material conveniently collected in one place (in addition to those mentioned the volumes include a number of other valuable articles) and will be an important source of references. Some subjects are, however, controversial—for example, the nature of heterochromatin—or complex—like repetitious DNA and its possible functions—and readers no doubt will feel a real need to consult the dozen or so annual reviews where related and sometimes more lucid articles can be found. Most chapters are very specialised and won't be suitable for undergraduate study.

There is no doubt that the books, which seem at first sight formidable in their bulk, would have benefited greatly from a good historical introduction outlining the important discoveries and showing how articles were chosen so as to fit into the general scheme.

Howard G. Davies

Plants and salts

Ion Transport and Cell Structure in Plants. By David Clarkson. Pp. xi+350. (European Plant Biology Series.) (McGraw-Hill: London and New York, April 1974.) £6.95.

THIS is the first book on plant-salt relations I have seen which takes full account of the advances in the subject which have been made in the last 10 years. In this respect it makes some recent American texts look distinctly old fashioned.

Here the undergraduate, for whom the book is intended, can read about isotope washout curves, the electrochemical approach and the chemiosmotic hypothesis, topics which have not been seen together before in a book of this type. The first six chapters are concerned with the cellular level and include an excellent chapter on the cell membrane. To illustrate the principles Dr Clarkson frequently refers to the alga *Hydrodictyon africanum* as a model; a good idea for an undergraduate text.

The second half of the book deals with the salt relations of the higher plant and in particular the root. It is not as well set out or as well written as the first half which one feels would make an excellent book on its own.

There are one or two errors in the text which might confuse the undergraduate such as the one on page 227 where in a discussion about the structure of the endodermis the reader is referred to a figure which turns out to be a scanning electron micrograph of a xylem vessel. Despite these blemishes Dr Clarkson is to be congratulated on his bold modern approach.

D. J. F. Bowling

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Phycology and physiology

Algal Physiology and Biochemistry. Edited by W. D. P. Stewart. (Botanical Monographs, vol. 10.) Pp. xi+989. (Blackwell Scientific: Oxford and London, 1974.) £15.50.

MUCH basic physiological and biochemical information on plants comes from the study of algae, and the general plant physiologist will be interested in this book as a summary of current knowledge on algal chemistry and function. The phycologist's requirement is also for a clear statement of the algal situation, together with a brief setting of the general scene so that cross comparisons can be made for major groups within the algae and between algae and other plants. That format is adopted by most of the authors contributing to this book, usually with marked success.

The book begins with a sensible comment on biochemical taxonomy, appropriately enough by R. A. Lewin who edited the 1962 *Physiology and Biochemistry of Algae*, now superseded by the present volume except as a survey of the earlier literature; I am glad to see Dr Lewin warn against the dangers of generalising from studies on one strain of one species under one set of conditions, and of making comparisons at class level from knowledge of a few species out of thousands. This is followed by comprehensive chapters on cell walls and polysaccharides, storage

products, fatty acids and lipids, sterols and nucleic acids. Each contains a wealth of information correlated to the biology and taxonomic position of the algae concerned. In a chapter on nuclear and cytoplasmic inheritance in green algae, Ruth Sager confines her account almost exclusively to investigations on *Chlamydomonas*. The result is a chapter of substance by the expert in the field and, since a forthcoming title in the Botanical Monographs series is a 250 page book on *The Genetics of Algae*, Dr Sager is obviously right to present this account rather than a telegraphic review of all algal genetics.

Unfortunately, one or two chapters attempt to summarise overlarge topics and fail for lack of space. In the chapter on cytoplasmic organelles, L. V. Evans tries to review the relationship between the structure and function of flagella, haptonemata, trichocysts, scales, lysosomes, microbodies, contractile vacuoles, walls, nuclei, microtubules, nuclear division, mitochondria and Golgi bodies, all in less than 20 pages of text. The task is manifestly impossible and the result is a catalogue of truncated descriptions and oversimplified generalisations that seem out of key with the rest of the book. Three pages are devoted to descriptions of mitotic ultrastructure in various algae but there is no comment on significance, either functional or phylogenetic. Golgi structure and function receive better treatment but it is a pity that a separate chapter could not have been given to this topic, allowing room for

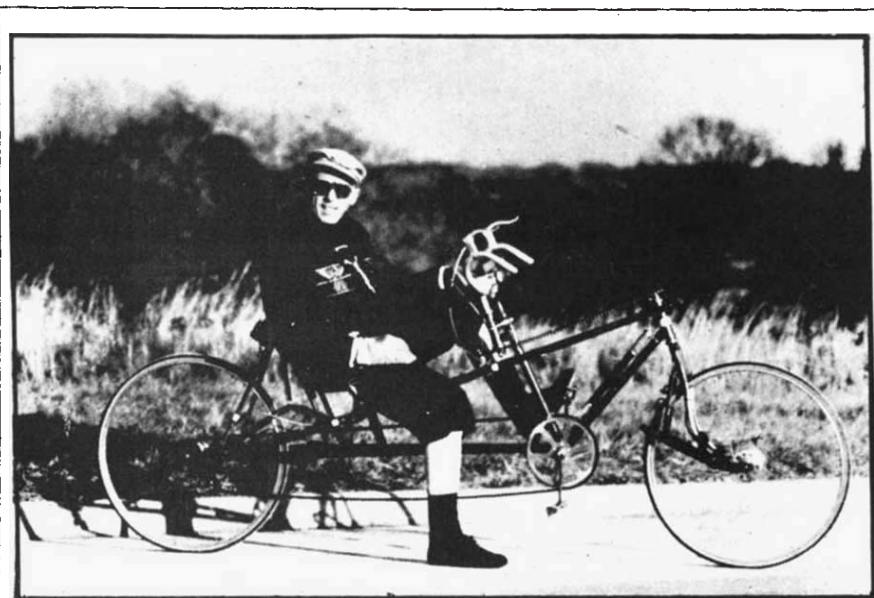
some interpretation and ideas in the account of other organelles. Plastids are given a whole chapter in which T. Bisalputra is able to discuss, albeit briefly, variations of chloroplast ultrastructure in relation to algal classes and pigments, and this is followed by chapters on chlorophylls, carotenoids and biliproteins, which satisfactorily tidy up many of the earlier conflicting and doubtful records (especially for carotenoids).

Moving from biochemical to physiological topics, we come to a series of excellent chapters on light absorption, emission and photosynthesis, photosynthetic electron flow—with a fascinating small follow-up chapter on the elucidation of the electron transport chain by the study of algal mutants—and a wide range of other topics. Physiologists may fight among themselves over some of the more controversial ideas expressed in these chapters but personally I found them, not surprisingly, highly informative and, more surprisingly for a non-physiologist, largely intelligible. In cases where the technical aspects of the subject are difficult or, frankly, beyond me, I still found that most of the authors were able to convey to me the general significance of the studies under discussion.

In a short review one can do little more than indicate the main coverage of the book and comment on its general standard. The coverage is wide, the general standard excellent. If there are any significant errors of chemical fact these will doubtless be commented on in other reviews by biochemists. There is a discrepancy between Evans (pp. 96-7) and Nultsch (p. 866) in the information on microtubule subunits, even allowing for the fact that $4.5\ \mu\text{m}$ is a misprint for $4.5\ \text{nm}$ in the latter account; Prasinophyceae are characterised by scaly flagella rather than hairy flagella (p. 3); other errors and misprints are few and far between. Production is good, though some of the electron micrographs are overenlarged, badly printed and chopped off at the edges. The price is high, but that is to be expected for a 1974 book of nearly 1,000 pages.

As a nonphysiological, nonbiochemical phycologist, I found some parts of this book easy and enjoyable to read and other parts heavy going. In relation to my research, teaching, reading and editing interests in the algae, the book has already proved informative and stimulating. The data summaries, formulae, tables and discussions will be invaluable for reference purposes. There is no doubt that this book will be an essential work of reference for years to come, both for the phycologist and the plant physiologist.

Gordon F. Leedale



Captain Dan Henry, supine upon his recumbent bicycle. Captain Henry, from Flushing, New York, became well known for his early design of sprung bicycle. His adaptation, though somewhat cumbersome by today's standards, rendered his machine "faster in hill climbing", and he was able to note with some satisfaction that his tyres lasted longer. Energy conservationists take note. From *Bicycling Science. Ergonomics and Mechanics*. By Frank Rowland Whitt and David Gordon Wilson. Pp. xiii+247. (MIT Press: Cambridge, Massachusetts, and London, 1974.) n.p.

Biochimie. Edited by Francois Chapeville and Hubert Clauser. Pp. 872. (Hermann: Paris, 1974.) Fr. 178.00.

THE appearance of a comprehensive new textbook of biochemistry in French may well be met by English-speaking biochemists with an equanimity verging ever on indifference. For all that, it is an event of no little note, for creeping Americanisation has been threatening increasingly the character and individuality of European science. In most areas of research the European languages have been all but displaced by the new *lingua franca*. Very recently a discovery in a French laboratory was given wide publicity in the press, and *Le Monde* thought it worth remarking, in a tone of apparent asperity, that the work was being published in the American journal *Proceedings of the National Academy of Science of the U.S.A.* (rather, by implication, than in the corresponding French organ), and in French.

That this comment should ring so quaintly, shows how completely the realities of the situation, unpalatable as they may be, have been accepted by French (and no doubt other European) scientists in recent years. If English is the currency of research, however, it must nonetheless not be allowed to become the exclusive language of science. If French science is to preserve its own distinctive flavour and personality, matured through centuries of tradition and evolution, the language must be kept alive in the science faculties and textbooks. That in itself seems to me good reason to be grateful to the publishing house of Hermann, which has followed its admirable series of silver paperback monographs with a substantial new text, edited by Chapeville and Clauser and comprising separate contributions from 11 authors.

The publisher has secured a distinguished line-up of contributors, who have provided a well-balanced coverage. The book deals with bioenergetics, reaction mechanisms, proteins and amino acids, enzymes and catalysis, structure and metabolism of the saccharides, lipids and steroids, nucleotides and nucleic acids, intermediary metabolism, oxidation, phosphorylation, photosynthesis, biosynthesis of metabolites, and the chemistry of heredity. The treatment throughout is authoritative and clear. Some of the authors lean to a conservative view of what constitutes the established canon, and tend, therefore, to stress the classical rather than the topical.

The book is altogether not free from the inevitable drawbacks of multi-author treatises; in particular one

misses the unifying thread that a single author can at best impose by his view of the subject. There are also places in which the fields of interest do not interlock. For example, there is no mention of muscular contraction, chromatin, or nervous conduction, and the word rhodopsin is not in the index. But these lacunae are almost universal in the standard text-books in English, and I do not wish to cavil with what strikes me as a notable achievement. All of the authors have long teaching experience behind them, and many

French versions

generations of undergraduates and graduate students (myself included) have sat at the feet of most of them. They seem to me to have fulfilled their tasks admirably. And though it would be invidious to draw comparisons my personal choice for style and stimulus would be the chapters written by the two editors.

The book is attractively laid out in the modern style, with elegant typeface and attractive diagrams in pastel shades, which I hope will now generally supplant the rather stately and oppressive format, too often characteristic of scholarly works in European languages. I do not doubt that it will benefit French undergraduates, medical students and research workers for many years to come, and one may hope that translation may also render it ultimately accessible to English readers.

Anne d'Albis

Geologie de la France. Vols 1 and 2. By J. Debeltmas. Pp. 1-294, vol. 1; 295-539, vol. 2. (Doin Editeurs: Paris, 1974.) Fr75.00 each.

THIS book is a series of articles written by various specialists, each of them working on one of the natural geological regions of France. In the preface, Jacques Debeltmas, who dealt with the coordination of the various articles, states the ambitions and limitations of his undertaking: "... the problem is not only to give a perspective of the stratigraphy, and the tectonics of the various regions, ... but to link both, so that it is possible to recognise a structural evolution through a succession of features and, on the other hand, to use this evolution when it is known, for a better understanding of these features through time and space ...". There is another requirement, conciseness: "... it is a mainly didactic introduction to the geology of

France". This is not a treatise, but more a manual of regional geology for the use of students, which excludes numerous discussions and hypotheses, and long bibliographical lists.

The book is divided into two volumes. In the first, after a general introduction to the geology of France, the reader will find a description of the "Ancient massifs" — the Ardennes, Vosges, Armorican Massif and Massif Central—and a study of the vast sedimentary basins—the Paris Basin and the Aquitaine Basin—in which the pre-Mesozoic structure is depressed although the secondary and tertiary layers are only slightly deformed. In the second volume, there is a description of the folded ranges of the Alpine cycle: the Pyrenees, Provence, the Franco-Italian Alps, Jura, Corsica, Burgundy and the Rhone valley and Languedoc.

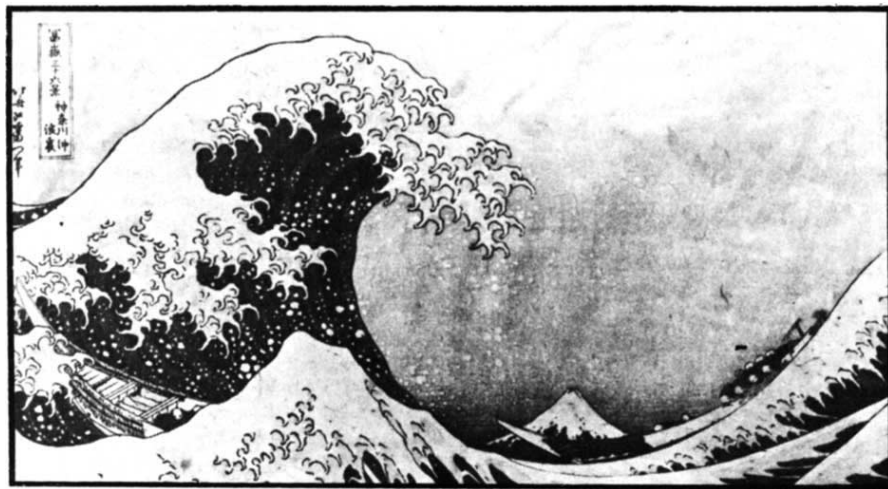
One is left with two impressions. First, the work has achieved part of its aims: because of its legibility, many illustrations, logical presentation, and also because of the care of the authors to reach the quintessence of their subjects, the book is both easy to read and interesting, and its success among students is assured. It is very convenient to have, assembled in one book, records of the large number of geological studies made in France since the last century.

The second impression which remained was of the disappointing lacunae within the book. All discussion of plate tectonics is set aside. Certainly the reader is made aware of this in the preface; but is it really defensible to omit a subject which has revolutionised earth sciences in the last few years? In addition, surface geology, easily observable, is highlighted at the expense of depth geology, which demands the indirect methods of geophysics; for example, no information is given on the thickness or structure of the crust. An analysis of seismology (which could have introduced a chapter on neotectonics), and of the interpretation of magnetic and gravimetric ideas, though essential to all structural studies, are missing. Finally, the presentation of the deep structural levels of the "Ancient massifs" does not pay enough attention to the recent results of microtectonics, the study of metamorphism (facies and facies series) and geochemistry of magmatic rocks.

This is an instructive and attractive book; but perhaps it will give students too incomplete a picture of the geology of France; and it may give foreign readers a false impression of French research in the earth sciences.

G. Boillot and R. Capdevila

Finding answers from the seas



The Sea: Ideas and Observations on Progress in The Study of The Sea. Vol. 5: Marine Chemistry. Edited by E. D. Goldberg. Pp. xiv+895. (Wiley-Interscience: New York and London, June 1974.) £21.20.

AT the First International Oceanographic Congress in New York in 1959, Lars Gunnar Sillén, Professor of Inorganic Chemistry at the Royal Institute of Technology in Stockholm, was invited to express the views of a laboratory chemist on the composition of sea water. His lecture, "The Physical Chemistry of Sea Water" represents a milestone in the development of ideas about the chemistry of sea water and the evolution of the composition of the hydrosphere, atmosphere and lithosphere over geological time. As Goldberg points out, the lecture was, in fact, the first of a series of investigations of the implications of equilibrium calculations of the ocean system, which challenged many prevailing notions and provided new ideas about a wide range of natural phenomena.

Volume 5 of *The Sea* is dedicated to Sillén and consists of accounts of the status of specific aspects of marine chemical research and includes chapters on completely new growth areas which have developed since Sillén's passing.

The status reports are largely contained in Part I (Thermodynamics of the Seawater System) and Part III (The Sedimentary Cycle). Millero, Distèche, Dyrssen and Wedborg review the purely thermodynamic aspects of sea water as an electrolyte solution. Gieskes discusses the alkalinity/ CO_2 problem, and confirms the confusion that arises over the use of apparent constants to describe the CO_2 system in the sea. That chapter is particularly refreshing because in addition to thermodynamic and kinetic considerations, mention is

made of the influence of both sediments and purely physical oceanographical processes on the distribution of CaCO_3 and some other chemical parameters in the sea. Breck reiterates his own suggestion that the pE (the redox potential) in oxic seawater is governed by the $\text{O}_2/\text{H}_2\text{O}_2$ couple and has a value substantially different from that estimated by Sillén.

Part III is by far the largest section of the book, and starts off with a chapter by Garrels (who specifically mentions the effect of Sillén's 1959 lecture on the direction of his own research) and Perry. It is a brief summary of the main conclusions of several papers by Garrels and his co-workers, and of the book *Evolution of Sedimentary Rocks* by Garrels and Mackenzie (1971). The section also has two interesting and quite different chapters on specific problems in sedimentary geochemistry, namely magnesium (Drever) and silicon (Wollast). Chapters on diagenesis (Berner), interstitial waters (Manheim and Sayles) and sulphur (Goldhaber and Kaplan) emphasise the large amount of attention currently directed towards post-depositional reactions in unconsolidated sediments, reactions which are important in controlling the long-term composition of sea water itself.

In Part IV (The Impact of Life Processes Excluding Man), Menzel gives a timely, if partisan, review of the mass of conflicting data on the distribution of dissolved and particulate carbon in the sea, and its metabolic fate. A large amount of data obtained about 10 years ago, which was treated with some scepticism when it first appeared, is apparently reliable so that some early, hotly debated, suggestions about the carbon cycle are probably correct. The relatively new area of the chemistry of marine natural products is reviewed in

depth by Faulkner and Andersen, and Lowenstam offers a lengthy account of the information available on the chemical composition of inorganic skeletal material and the impact of skeleton building on other chemical and physical processes in the oceans.

Part V (The Impact of Man on the Chemistry of the Oceans) is remarkably short in view of current interest (albeit concern) in the topic and the influence that the editor has himself had in drawing attention to it. The chapter by Jernelöv on heavy metals, metalloids and synthetic organics is very brief and inadequately referenced, and that by Preston dealing with artificial radioactivity is simply an inventory and a straightforward account of the possible behaviours of important radio-nuclides. Part VI (Origin of the Ocean) contains a single chapter by Arrhenius, De and Alfvén, which reviews their own recent work in several far flung branches of space research.

The most interesting contributions in this large volume are, however, contained in Part II (Air-Sea Interactions). They are interesting because they are concerned with new growth areas in marine chemistry. Thus, MacIntyre presents an iconoclastic, in places amusing, and thorough review of reactions in the sea-surface microlayer using a mass of data drawn from sources not immediately available, or indeed obvious, to marine scientists. Seiler and Schmidt, in a refreshing account of non-conservative gases in the sea, point out that the main source of nitrous oxide in the atmosphere is in fact the ocean, and that the atmospheric budgets for hydrogen, methane and carbon monoxide are affected to important degrees by processes in the sea.

This volume, although it is an invaluable addition to the series, does have some deficiencies. Specifically, the success or otherwise of the ion association and specific interaction models in describing the properties of sea water electrolytes is passed over rather swiftly. Recent work by Whitfield, Leyendekkers and Pytkowicz, for example, has given interesting new insight into the utility of some of the available models. A second almost completely neglected area which is only mentioned somewhat obliquely by Gieskes and Menzel, concerns the currently popular use of advection-diffusion models to describe and to understand the distribution of certain chemical parameters in the oceans. Whatever one may think of the usefulness of this particular approach, it has focused some much needed attention on some of the hidden effects that physical and biological processes have on the behaviour of some of the variables frequently measured by marine chemists.

S. E. Calvert

Deep Sea Sediments. Edited by Anton L. Inderbitzen. Pp. ix+497. (Plenum: New York and London, 1974.) \$42.00.

THIS volume, resulting from the proceedings of a symposium conducted by the Ocean Science and Technology Division of the US Office of Naval Research in 1973, is remarkably comprehensive. The 23 papers achieve more than adequately the purpose of the book, to bring into sharper focus the "state-of-the-art" regarding the physical and mechanical properties of sediments and the need to establish some degree of coordination among investigators. What has had to be left out has been amply included in the references at the end of each paper which, in total, comprise a truly universal bibliography in the field of sediment mechanics.

From a panoramic viewpoint the volume deals mainly with the present situation regarding sampling devices, laboratory measurements (both mechanical and physical) and the extension of laboratory investigations to the field.

In the Atlantic, Indian and Pacific Oceans, physiographic provinces have been devised as a means of establishing some order to the quantity of mechanical data which is becoming available. Empirical equations relating mechanical and physical properties have shown minute spatial variations within each province, and carbonate distribution studies may explain some of these variations. It is, however, stressed that the provinces are not homogeneous entities and that although the mechanical/physical correlations may indicate trends, overall correlations are less accurate than those obtained for single provinces.

A number of papers deal with an extension of the correlations from laboratory/laboratory to laboratory/field, and serve to indicate that, at present, the prediction of *in situ* sediment properties and of the settlement of structures to be placed on the sea floor are woefully inadequate, with up to 30% error under ideal conditions of measurement.

Therefore, questions are posed concerning the measurements being taken. Is the macroscopic approach of the engineer good enough? Shall we ever achieve good all-round working prediction models using these macroscopic methods? The safety factor of the engineer conceals a lot of what is lacking in theory. Thus, it seems that more effort is required in a study of microstructure—so that the engineer's methods may become modified for the sediment researcher.

The urgency of the situation is stressed amply in these papers which indicate the imminence of commercial enterprise in the mining of ferromanganese deposits as a source of

Natural Gases in Marine Sediments. Edited by Isaac R. Kaplan. Pp. viii+324. (Plenum: New York and London, 1974.) \$30.00.

THIS volume consists of 17 chapters, which originally derive from papers presented at a symposium held at Lake Arrowhead in November 1972. The various possible sources of gas in sediment are discussed in the early chapters, together with such analytical information as is available. Because of sampling difficulties, most of the data refer to shallow water cores or to certain Deep Sea Drilling Project sites; by now, much of this information has been available in the literature for some time, but it is useful to have it presented in one volume, if only because it emphasises the restricted range of data available.

The latter half of the book is given over to consideration of the physical state of the gases in deep sea sediments, with particular regard to the possibility that gas-water clathrate compounds, or 'gas hydrates', may occur under the *in situ* conditions of low temperature and high pressure. Three chapters on the known thermodynamic properties of such compounds establish the areas of the world ocean in which these ice-like solids are stable; and as gas concentrations must be very high, some mechanisms, such as the *in situ* production of methane by the bacterial decomposition of organic material, or the injection of carbon dioxide, hydrogen sulphide, or methane from geothermal vents, must also be available.

Given that, the possible zone of hydrate occurrence is bounded on the upperside by the increase of temperature and decrease of hydrostatic pressure as the sea surface is approached from below and on its lower side by the increase in temperature

resulting from the geothermal gradient. For instance, in sea water which is 2 km deep, and which has a bottom temperature of 2 °C, it is shown that methane hydrate would be stable to a depth of about 600 m within the sediment. Although the hydrate would theoretically also be stable in the water column above the sediment at all depths below about 500 m, it is not likely that the required gas concentrations could be sustained outside the sedimentary column. Thus, the phenomenon is to be expected only within the upper regions of deep sea sediments.

Although no unequivocal proof of the existence of gas hydrates is given, three chapters describe the occurrence of sub-bottom acoustic returns which could be interpreted in such terms. Two authors also describe laboratory rigs for the formation and study of hydrate-sediment mixtures, and preliminary results are given.

One editorial function, which is particularly difficult with symposium volumes, is to ensure that a subject is covered evenly and without unnecessary repetition. The editor of this volume has included a useful and well written introduction which draws together the threads of research presented by the various contributors; it is a pity that he could not at the same time have eliminated redundancy. It is, for instance, irritating to be told five times of Humphry Davy's discovery of chlorine hydrate. The appearance of the book, apparently produced by a photo-offset process from a very superior, accurate and uniform typescript, is beyond reproach. *Natural Gases in Marine Sediments* can be recommended to all those interested in marine sedimentation, as a useful introduction to an under-explored field of study.

T. R. S. Wilson

copper, nickel and cobalt and which consider the resulting public concern over the protection of fauna and environment.

There is a need for better instruments for obtaining undisturbed samples. Box covers seem to give good samples but are at present limited to the near surface (36 cm). There is, however, an even greater need for undisturbed material from greater depths. The transport of core material, as well as the actual sampling procedure, produces varying degrees of disturbance. That, and the weakness of prediction models, has stimulated more *in situ* work.

Yet the contributors have not forgotten that their research is often

limited by economic stringency and that laboratory work relating to the deep sea environment must always play a vital role.

It is, however, pointed out that economic constraints can be lessened by means of a coordinated approach to sediment research.

There is a call also for the standardisation of techniques, symbols and units of measurement, concerning which this volume has put forward many worthwhile proposals. Standardisation would necessarily save much time and confusion.

The bibliography, data, problems and ideas presented in this volume will be invaluable for future work in sediment mechanics.

Sinclair Buchan

Living ancient history

Introducing Geology: The Earth's Crust Considered as History. By D. V. Ager. Pp. 256+21 plates. Second Edition. (Faber and Faber: London, February 1975.) £3.95, hard bound; £1.75, paperback.

THIS is a book about very ancient history. It reviews, in readable language, the geological evolution of the British Isles, with some references to other parts of the world. Since the first edition appeared in 1958, there has, as everyone knows, been a revolution in the earth sciences: continental drift has now been proved, and seafloor spreading, subduction and plate tectonics have all become recognised as aspects of the surface layers of a planet that is probably expanding. This new edition adds a bold but sufficient outline of these exciting developments to the first chapter and applies them at later stages; for example, the mid Palaeozoic troughs are seen as resulting from the opening of a proto-Atlantic between plates bearing America and Europe on their backs; plates which later closed to produce the Caledonian Mountains. Similarly, the break-up of the former southern continent of Gondwana under the influence of convection currents in the upper mantle is used to illustrate an important stage in geological history. In that and other ways, the reader is introduced to modern ideas of global tectonics.

The general treatment remains the same: the consideration of groups of geological epochs and systems. Thus, the chapters entitled "Oldest Rocks of All" covers the 4×10^9 years of Precambrian time; the "Oldest Rocks with Fossils", the Cambrian, Ordovician and Silurian systems; "Dry Land and Shallow Seas", the Devonian and Carboniferous. Each chapter is illustrated with maps showing the general outcrops of the relevant strata in Britain; and palaeogeographical diagrams, some original, some based on the works of L. J. Wills and F. W. Shotton, are included. There are illustrations of typical fossils but there is a minimum of fossil names and almost no mineral names. The chief rock-making and physiographical processes are described as they become relevant and they are illustrated with well produced plates, prepared mainly from Geological Survey photographs.

In one respect, Professor Ager has again been overtaken by events. His chapter entitled "The Long Quiet Episode", devoted to the Rhaetic, Jurassic and Cretaceous, was very suitably named when he prepared this second edition; as he says, it was reason-

able to think of the European plate sailing peacefully at this time. But last November, the North Sea petroleum operators released their geological results, showing that the later stages of Jurassic time were not all quiet in the British area. There were in fact major rift-forming movements in the centre of what is now the North Sea, accompanied by modest volcanic activity and followed by extensive erosion. The rift profoundly affected Cretaceous sedimentation, which had filled up the trough by the time that the Tertiary basin covered it. The effects of these 'Kimmerian' movements explain the absence and reappearance of Jurassic strata in various parts of the North Sea Basin, and make us realise that the apparent continuity of the Jurassic outcrop across England is no indication of what happens in the third dimension. There will be material for a third edition of this book, but in the meantime it is none the worse for having missed the Kimmerian. Geology is, after all, a living and developing subject, and this historical approach to the country which so much influenced its progress is a good introduction to it.

Kingsley Dunham

By jove

The Jupiter Effect. By John Gribbin and Stephen Plagemann. Pp. 136. (Macmillan: London and Basingstoke, 1974.) £3.95.

"... all we have done is to gather in one volume data from scattered sources and look at them anew ..."

And a splendid job the authors have made of weaving a complex, yet neat, design from the geophysical observations they have used. There may be a piece or two still missing, and the picture might well fall apart when lifted by the corner, but nonetheless it is a readable yarn which inquisitive citizens will enjoy; and seismologists may envy the authors' courage in undertaking to predict that the next great earthquake on the San Andreas Fault will occur in 1982, give or take a couple of years.

In that year the planets of the Solar System will be in conjunction with every other planet for the first time in 179 years. On the evidence of correlations—observed since the 17th Century—between peak sunspot activity and peak tides raised on the Sun, the authors predict a dramatic increase in activity for 1982. This, they argue might initiate a chain reaction through the interaction of solar wind and cosmic rays with the upper atmosphere, thereby affecting world wide atmospheric circulation patterns which in turn

would change the Earth's rate of spin to provide the relatively small triggering force required to raise the 'normal' level of seismicity in highly strained belts. Observational evidence is well documented, and the circumstantial links in the chain plausibly argued. In the process the authors have provided lay readers with an educational glimpse of several geophysical processes and the geophysicist with sufficient stimuli to follow their suggestion to "wonder how such an interaction *could* work".

Why pick especially on the San Andreas Fault? Well for one thing it must attract wider interest (and a larger market) than, say, the Highland Boundary Fault and for another it is so well studied, so closely knitted instrumentally, that even the seismologists concerned are confident enough to venture predictions (though none so closely as 1982 ± 2 years). The latest of these professional forays into the fashionable field of earthquake prediction was published in *Nature* a few months ago and rules out the area between San Francisco and Parkfield for a really large earthquake before 1999, but accepts the possibility of a moderately large disturbance from 1981 onwards. Those predictions were based on recent observations of the rate of change of P-wave velocities, on which readers are brought up to date in an appendix to *The Jupiter Effect* (a title which neatly fits a book that unashamedly and provocatively attempts a geophysical explanation for the predictions of astrologers). Gribbin and Plagemann did not have time to fit this more recent work into their jigsaw and, in any case, they persuade us by other published evidence that the area on the San Andreas which is primed for triggering lies nearer to Los Angeles, south-east of Parkfield.

Even if such forecasts turn out to be less precise than hoped for, there is little doubt that California, as well as other thickly populated seismic areas, will suffer from great earthquakes which cannot be avoided unless lubrication can be applied to smooth the rate of release of stored energy. And as it would be a very brave (or ill judged) decision to pump water along the San Andreas Fault, the authors return to the unglamorous duty of spelling out the precautions which town planners and individuals can take to mitigate the inevitable effects. That their common sense statements of the obvious are considered worth printing demonstrates the social nature of the problem. The most sophisticated of observational and analytical efforts will contribute nothing to ease the disaster confronting a dam which, on the face of it, is intended to staple the edges of the Pacific and North American plates.

H. I. S. Thirlaway

(Continued from page 212)

as previously thought, a more or less accessory effect of extracellular secretion. Endolithic cyanophytes may even turn out to be 'rock-eaters' in the true sense.

Scanning electron microscope (SEM) studies of borings of a filamentous blue-green alga of the *Hormatonema* form¹⁻³ revealed the marks of a precisely controlled excavating process, suggesting the existence of specialised boring organelles in the alga (Fig. 1). Indeed, the discovery in the cyanophytes of very refined adaptations to an endolithic mode of life should hardly be a surprising find, since these organisms, according to palaeontological data, have been boring into calcareous substrates at least since the early Ordovician (that is, for about 500 Myr) and perhaps much longer than that^{4,5}.

The boring mechanism of endolithic algae has previously been described as an extracellular dissolution process: acid or chelating fluids, released by the terminal cell, supposedly dissolve small volumes of the mineral substrate and the algal filament penetrates step by step into a tunnel formed by a sequence of small hollows^{3,6}. How the fluids are removed from the inner end of the inhabited tunnel, whether by extracellular circulation/diffusion or by metabolic uptake and intracellular transport, is not known. In the dissolution process, the micromorphology of the excavated space is determined by the solubility characteristics of the bored substrate and is thus beyond the immediate control of the organism. For example, the void space in bored calcite spar replicates the calcite rhomb⁶, and borings in dolomitic limestone may contain residual crystals of poorly soluble dolomite which has resisted the attack⁷.

The material described here derives from saline rock pools in the upper swash zone in modern beachrock west of Stazousa Point on the northern coast of Cyprus (see ref. 8). The bored rock consists in part of a coarse crystalline limestone, with optically uniform calcite crystals, several hundred micrometres across. These crystals provide excellent substrates for the study of algal borings by means of SEM.

The endolithic algae consist of dark blue filaments, about 300 μm long and 10 μm in diameter. Exposed to acid solutions the dark blue pigment turns reddish-brown, a reaction considered typical in *Hormatonema*^{3,6}. In isolated colonies consisting of a few filaments, the algae grow radically away from a common starting point whereas in crowded colonies parallel filaments grow perpendicular to the rock surface. These arrangements are consistent with the observation that algal filaments commonly repel each other and avoid connecting with other tunnels⁹.

The cavity formed by an individual filament is a fairly straight tunnel, replicating the organism. Branching of tunnels has not been observed.

As reconstructed from the microtopography of the tunnel walls, the boring process proceeds through the etching out of small grooves, about 1 μm in width and depth and 10 μm in length (Fig. 1). The wall relief increases slightly behind the terminal cell. The orientation of the grooves is approximately at right angles to the long axis of the tunnel. Interestingly enough, there are two laterally opposite sets of grooves, meeting in a zone of small pyramids. The arrangement indicates a bilateral symmetry in the boring apparatus.

The pattern of grooves shows no relationship to the internal ultrastructure or to the crystallographic parameters of the bored substrate. For example, where several tunnels run at different angles within a single calcite crystal, each tunnel has its own set of grooves which are perpendicular to the tunnel axis and are not related to the crystallographic structures of the continuous calcite lattice.

The bored rock material was collected for geological purposes and the samples were only rinsed in distilled water and air dried. Because of this crude treatment, the material

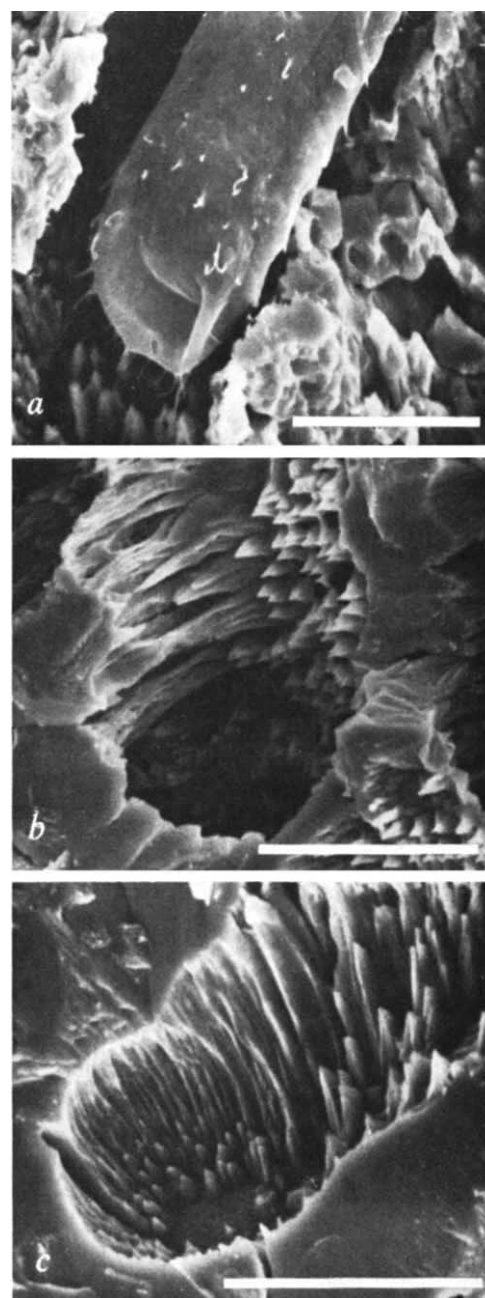


Fig. 1 Cyanophyte borings in calcite: SEM micrographs of fractured material. *a*, Penetrating tip of algal filament, slightly withdrawn from the inner end of the tunnel by shrinkage during preparation (note threads on the filament surface); *b*, oblique fracture cutting four algal tunnels in homogeneous calcite, showing one side of the bilateral pattern of grooves and the zone of small pyramids; *c*, inner end of algal tunnel with grooves and pyramids. Scale bars 10 μm .

is not suitable for a study of soft tissues, and the marks of the boring process cannot be related to specific parts of the algae. Complete algal cells, about 15 μm long and 10 μm in diameter, can obviously be ruled out as direct operators, but identification of possible boring organelles must await properly fixed and dehydrated algal material.

It is, however, interesting to observe that even in this poorly preserved material the algal filaments have a sparse coat of small threads, about 0.2 μm in diameter and 2–6 μm long (Fig. 1*a*). Similar threads have not been noted in the rich assemblages of endolithic algal filaments found in marine calcareous sediment grains^{6,9-12}. Although there is

no real evidence to suggest that the threads are boring apparatus, their presence here is probably significant.

Three conclusions can be drawn from the micromorphology of the borings. First, excavation is a chemical process, but is not straightforward extracellular dissolution on a whole-cell scale. Second external organelles, about 1 μm wide and 10 μm long, are the immediate operators. Third, the boring apparatus has a bilateral symmetry.

It can be added that, although this article considers a single species of *Hormatonema* at one locality only, I have observed similar borings in the course of SEM analyses of sedimentary carbonates from the North Sea and the Baltic Sea. It seems probable that the described mode of boring is common in the cyanophytes.

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Correlation between selenium and mercury in man following exposure to inorganic mercury

THE ability of selenium compounds to modify profoundly the toxicity of both organic and inorganic mercury compounds has been demonstrated in experimental animals by Parizek and co-workers^{1,2}. The analytical data of Ganther *et al.*³ on tuna and of Koeman *et al.*⁴ on marine mammals showed that natural levels of mercury and selenium are strongly correlated. Here we report an approximately molar ratio for these elements in certain human organs following exposure to high levels of inorganic mercury.

Mercury in post-mortem samples from three groups of people—workers in the mercury mine at Idrija, Slovenia, inhabitants of the town of Idrija and a control group with no known exposure—has been analysed over the past three years^{5,6}. The study has been extended to include selenium. Although we do

not intend to discuss the mercury levels in detail, some interesting findings emerged (Table 1). Notably, the high accumulation and retention of mercury in the thyroid and pituitary; in the miners increases are of the order of a thousandfold, and in the population group about tenfold, relative to the controls. The kidney has previously been considered to be the prime accumulator of inorganic mercury, but in all eight mine workers, it ranked only third. Seven of them had been in retirement for periods up to 16 yr before death (see legend to Table 2), yet the pattern of distribution was essentially similar. It is clear that the retention of mercury in the organs with the highest accumulated levels, namely, thyroid, pituitary, kidney and brain, is very great.

In more recent post-mortem samples we have analysed both selenium and mercury to find a possible relationship between these elements in exposed humans. Both elements were analysed simultaneously by neutron activation from the same sample aliquot, using a volatilisation technique⁷ modified to allow separation and measurement of both mercury and selenium⁸. The results to date are shown in Table 2, and clearly demonstrate an approximately 1:1 molar ratio for those organs which accumulate and retain mercury strongly, namely, thyroid, pituitary, and kidney. The same effect is also seen in brain samples (Table 3), with different sections of the same brain all displaying a near molar ratio. Since selenium, as an essential trace element, will normally be present in at least typical physiological levels, whereas mercury in non-exposed persons should only approach insignificant amounts, a molar ratio will only be observed for rather elevated values. Even slightly increased mercury levels, however, seem capable of raising the selenium content; the ratio of the increments over normal levels approaches the molar ratio in many cases.

The correlation coefficient *r* between mercury and selenium in the organ group thyroid, pituitary, kidney and brain (subject T.A.) from the miners is 0.998, with two virtually identical regression equations (*r* very nearly unity) of the form

$$(\text{Se})\text{p.p.m.} = 0.41(\text{Hg})\text{p.p.m.} + 0.21 \text{ p.p.m.}$$

In molar terms, the ratio Hg–Se is 0.96:1. This ratio in the liver of marine mammals⁴ was near 1:1 in molar terms over a 2.5 decade mercury concentration range, but only a small fraction of the mercury could be recovered as methylmercury. In our work, methylmercury levels in the exposed groups, as determined by an isothermal distillation and gas chromatographic method⁹, were unremarkable, exceeding normal values only slightly. This also confirms the absence of any significant *in vivo* methylation in man.

The 1:1 molar ratio naturally suggests a direct Hg–Se linkage, though at this stage we can only speculate about the nature of the group or complex and its mode of attachment. Keeping in mind that the time between death and termination of exposure in the professionally exposed subjects varied greatly, it seems that the effect is both accumulative (as in the case of marine mammals and tuna) and retentive. This emphasises the strength of the Hg–Se interaction and suggests their removal from biological turnover. The coaccumulation of selenium observed in this study is certainly not the result of coexisting high levels of

Table 1 Average mercury content with standard deviation of human organs in p.p.m. fresh weight

Group	Thyroid	Pituitary	Kidney	Liver	Lung	Brain
Mercury mine workers	35.2 ± 28.5 (8) 7.8–101	27.1 ± 14.9 (7) 13.8–64.3	8.4 ± 4.9 (8) 2.3–18.5	0.26 ± 0.25 (8) 0.04–0.79	1.11 ± 0.89 (5) 0.13–1.58	0.70 ± 0.64* (6) 0.18–1.50*
Idrija population	0.70 ± 0.45† (10) 0.03–3.6	0.46 ± 0.54 (11) 0.02–1.76	0.66 ± 1.13 (11) 0.03–4.0	0.107 ± 0.059 (11) 0.02–0.21	0.127 ± 0.100 (10) 0.005–0.24	0.038 ± 0.045† (9) 0.002–0.11
Non-exposed controls	0.030 ± 0.037 (16) 0.003–0.13	0.040 ± 0.026 (6) 0.007–0.064	0.14 ± 0.16 (7) 0.01–0.37	0.030 ± 0.017 (8) 0.01–0.05	—	0.0042 ± 0.0026 (5) 0.001–0.007

Figures in parentheses refer to the number of subjects analysed, ranges are given below.

* Excluding T.A.; see Tables 2 and 3. † Excluding P.M.

Table 2 Mercury and selenium in human organs in p.p.m. fresh weight

Subject	Thyroid			Pituitary			Kidney			Liver	
	Hg	Se	Hg/Se	Hg	Se	Hg/Se	Hg	Se	Hg/Se	Hg	Se
Mine workers											
F.M. (m)	7.8	3.2	2.4	14.5	6.1	2.4	7.5	2.5	3.0	0.36	0.38
J.V. (m)	29.7	12.6	2.4	13.8	6.4	2.1	6.0	2.1	2.8	0.37	0.40
B.P. (m)	26.5	12.3	2.2	27.7	13.3	2.1	2.3	1.6	1.5	0.037	0.36
T.A. (m)	101	41.1	2.5	47.9	—	—	11.4	5.1	2.2	0.065	0.38
Z.F. (m)	5.0	1.5	3.3								
Idrija population											
P.M. (f)	14.4	5.72	2.5	0.59	0.39	—	0.76	0.70	—	0.067	0.21
D.I. (f)	0.026	0.16	—	0.020	0.34	—	0.61	0.47	—	0.085	0.12
Controls											
D.J. (m)	0.039	0.79	—	0.057	0.45	—	0.37	0.82	—	0.039	0.19
K.H. (f)	0.0023	0.46	—	0.044	0.34	—	0.011	0.54	—	0.011	0.17
Miners:	F.M. exposed 17 yr, 5 yr in retirement			B.A. 5 yr in retirement							
	J.V. exposed 21 yr, 5 yr in retirement			O.A. 10 yr in retirement							
	B.P. exposed 33 yr, 16 yr in retirement			P.A. 0 yr in retirement							
	T.A. exposed 29 yr, 16 yr in retirement			L.F. 2 yr in retirement							
	Z.F. surface worker (welder), 14 yr in retirement										
Population:	P.M. aged 82 yr, life-long Idrija resident (son a miner)										
	D.I. 4-month-old infant										

Table 3 Mercury and selenium contents of human brain in p.p.m. fresh weight

Sample	T.A.			B.P.		P.M.	
	Hg	Se	Hg/Se	Hg	Se	Hg	Se
Cerebellum	2.42	1.38	1.8	0.15	—		
	2.34	0.93	2.5				
Striatum and cortex	13.2	6.05	2.2			0.071	0.17
	2.96	1.43	2.1				
Thalamus	8.16	3.33	2.5				
Medulla oblongata	2.66	1.15	2.3				
Hypothalamus				0.10	0.17		
Substantia nigra				0.073	0.17	0.020	0.15
Occipital cortex				0.18	0.23	0.24	0.25

selenium in the mine environment, as analysis of dusts and soils showed low values (around 1 p.p.m.) compared with mercury which is in the 100 p.p.m. range. In marine mammals and tuna the diet is much more enriched in selenium relative to mercury.

Thus in general it seems that the effect can occur where mercury is present as the methyl (tuna) or the inorganic form (marine mammals and man), and whether selenium is relatively abundant in the diet or not. The protective effect of selenium in animals has been shown for various forms of mercury^{1,2}. It may be therefore that the coaccumulation of selenium is a natural or autoprotective effect. In this context it is interesting to note that possible preventive and therapeutic uses of selenium against mercury intoxication have been discussed by Parizek^{1,2}. Certainly, the detailed metabolic, dynamic and structural mechanisms of this phenomenon seem worthy of further intensive study, in view of its toxicological implications and relevance to the metabolic role of selenium.

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Genetic analysis of behavioural responses to novelty in mice

To trace the genetic determinants of exploratory behaviour in house mice, I started a programme of selective breeding¹. Using the F₂ generation from a cross between the inbred strains C57BL/6J and DBA/2J as the foundation population, male mice were selected bidirectionally for frequency of exploratory rearing responses displayed in a novel environment. To transfer alleles responsible for high rearing scores from the C57BL strain to the DBA genetic background, selection was combined with repeated backcrossing to DBA females over five consecutive generations. A comparable procedure has been used by Chai² for body size in mice. Since these early stages of the selection aimed at developing two lines of mice with similar genetic backgrounds, but differing for loci affecting rearing behaviour, no unselected control line was bred; the DBA strain could serve as this.

In the next stages, selection was continued with concomitant inbreeding by sib-mating to fix the relevant alleles within the two lines. Replicate selected lines^{3,4} were not bred. This does not seem too serious a drawback because the purpose of the experiment was to identify individual polygenes controlling exploratory behaviour and the physiological mechanisms through which they act, not to study the potential response to selection *per se*. In the course of this breeding process, a number of the relevant alleles may not be maintained in the lines because of genetic drift (see also Schlager⁵, who selected mice for blood pressure level).

After more than twenty generations of inbreeding, the lines, symbolised SRH (high) and SRL (low), must now be close to

Table 1 Raw data for rearing responses and locomotor activity in mice from different generations and genetic analyses under the assumption of monogenic control

Population	<i>n</i>	Mean	Variance	<i>n</i> rating $\geq$ critical score*		χ^2	<i>P</i> >
				Observed	Expected		
Rearing							
P ₁ (SRH)	20	44.25	489.88	16	—	—	—
P ₂ (SRL)	20	14.15	42.56	3	—	—	—
F ₁	30	32.80	145.48	23	—	—	—
F ₂	30	27.47	207.70	16	18.67	1.01	0.30
B ₁	30	38.77	201.49	26	23.55	1.19	0.20
B ₂	30	24.87	188.12	14	13.80	0.01	0.90
Total†	90	—	—	56	56.02	~0.00	0.99
Locomotion							
P ₁ (SRH)	20	95.20	1768.27	17	—	—	—
P ₂ (SRL)	20	51.55	773.52	8	—	—	—
F ₁	30	50.50	524.12	18	—	—	—
F ₂	30	61.90	1200.02	19	18.38	0.06	0.80
B ₁	30	71.80	1208.51	21	21.75	0.09	0.70
B ₂	30	57.07	1285.99	16	15.00	0.13	0.70
Total†	90	—	—	56	55.13	0.04	0.80

*For rearing, 25; for locomotion, 50.

†F₂ + B₁ + B₂.

complete homozygosity and they are still clearly different for rearing score. This is really a difference in the response to novelty, since in a separate experiment with 44 SRH and 46 SRL mice we failed to find any differences between the lines for various exploratory activities carried out in the home cage. The lines seem to differ also for locomotor activity measured in a novel environment¹.

To estimate the number of loci contributing to these behavioural differences, intercrosses (F₁ and F₂) and backcrosses (B₁ and B₂) were made with the two selected lines. This report deals with the results of the analysis of these hybrid types, applying in the first instance methods that have been used with behavioural phenotypes such as susceptibility to audiogenic seizures⁶, vocalisation⁷, taste sensitivity to PTC⁸, saccharin preference⁹, and passive-avoidance performance¹⁰ of mice. Single-locus inheritance was indicated and, to confirm this, the next step was a biometrical genetic analysis according to methods described^{3-5,11}. The relationship between rearing frequency and locomotor activity was also considered.

Female mice from the SRH strain (20th generation; P₁) were mated with males from strain SRL (20th generation; P₂) to produce an F₁ population (no reciprocals), and the F₂, B₁, and B₂ populations were bred from F₁ females. All neonate animals were fostered to nursing mothers from a random-bred stock, in conditions described previously¹. At the age of about 13 weeks, single male mice were placed for 15 min during the afternoon in a novel environment, an observation cage measuring 133 × 53 × 58 cm and with a bedding of peat dust. The frequency of rearing responses (vertical activity) and the frequency with which animals intersected light beams activating photocells (horizontal activity) were recorded on counters. The numbers of subjects observed were 20 (P₁, P₂) and 30 (F₁, F₂, B₁, B₂).

The left-hand part of Table 1 shows the untransformed behavioural scores. The non-segregating populations differed from each other for rearing: there was a significant strain difference ($P < 0.001$; Mann-Whitney *U* test), whereas the F₁ took an intermediate position ($P < 0.03$ and 0.001 , respectively). The latter was not true for locomotion; although the parental strains were clearly different ($P < 0.001$), the F₁ differed significantly from the P₁ only ($P < 0.001$) and it resembled the P₂. As far as the distribution of scores is concerned, particularly that of the B₂ suggested bimodality, indicating single-locus control of the behavioural differences. In particular with regard to rearing, the presumptively heterozygous animals within the pertinent populations seemed to have an optimum score (between 25 and 30). On the basis of the numbers of animals from the non-segregating groups that exceed an arbitrary critical score, we can calculate expected values for the segre-

gating populations and compare these with the observed numbers by means of χ^2 -square tests for goodness of fit, as shown in the right-hand part of Table 1. The observed values did not deviate significantly from those expected and, thus, the hypotheses of monogenic inheritance of rearing frequency and of locomotor activity score are not rejected.

A biometrical genetic analysis was also performed; judged by the variances of the raw scores of the non-segregating P₁, P₂, and F₁ populations entered in Table 1, however, significant genotype-environment interactions were present, that is, Mather's second criterion was not fulfilled (*F* tests; for rearing: $P < 0.001$, 0.005 , and 0.005 ; for locomotion: $P < 0.05$, 0.005 , and 0.20). A square-root transformation proved unsatisfactory, but transformation to natural logarithms effectively eliminated the genotype-environment interactions (see Table 2). In these transformed data, significant strain differences were also found for rearing ($P < 0.001$; Student's *t* test) and locomotion ($P < 0.001$), and the F₁ was again intermediate for rearing ($P < 0.02$ and 0.001 , respectively) but not for locomotion (significantly different from the P₁ only; $P < 0.001$). Application of scaling tests A, B, C, and D (Mather's first criterion) in Table 2 shows that the ln transformation rendered the scale of measurement adequate for rearing as well as locomotion, that is, any epistatic interactions were removed, as is also evident from the estimates of components [*i*], [*j*], and [*l*]. With regard to both behaviours, the additivity component [*a*] was significant and dominance [*d*] not significant. The heritability, calculated from the components of variation, differed from zero only for rearing (66%) and the minimum estimates of the number of effective loci yielded 0.71 and 0.58, respectively; these values are compatible with single-locus control. Some caution, however, is justified in view of the significant additivity components.

From the correlation coefficients in Table 3 it seems that rearing and locomotion are correlated, even more strongly in the segregating populations than in the non-segregating groups. Significant positive correlations were also established in most of the previous generations of the selection lines; it now seems highly improbable that these resulted from chance fixation of different genes. If traits vary dependently, as shown here for rearing and locomotion, the observed differences

Table 2 Transformed (ln) data for rearing responses and locomotor activity in mice from different generations and results of the biometrical analyses

Population	Rearing		Locomotion	
	Mean	Variance	Mean	Variance
P ₁ (SRH)	3.70	0.18	4.45	0.24
P ₂ (SRL)	2.56	0.19	3.82	0.27
F ₁	3.43	0.12	3.80	0.29
F ₂	3.17	0.34	3.97	0.38
B ₁	3.59	0.15	4.17	0.22
B ₂	3.07	0.31	3.83	0.58
Parameter	Estimate	s.e.	Estimate	s.e.
Scaling				
A	-0.05	0.18	-0.08	0.23
B	-0.15	0.23	-0.03	0.32
C	0.44	0.47	0.01	0.52
D	0.32	0.25	0.05	0.28
Component				
<i>m</i>	2.49*	0.50	4.03*	0.56
<i>a</i>	0.57*	0.07	0.32*	0.08
<i>d</i>	1.78	1.15	-0.003	1.35
<i>i</i>	0.64	0.49	0.11	0.56
<i>j</i>	-0.09	0.28	0.05	0.36
<i>l</i>	-0.84	0.68	-0.22	0.83
Variation				
V _A	0.23		0	
V _D	0		0.14	
V _E	0.12		0.29	
Heritability				
<i>h</i> ² _(b)	0.66*	0.12	0.36	0.23
<i>h</i> ² _(n)	0.66*		0	
Effective factors				
<i>k</i>	0.71		0.58	

*Significantly different from zero ($P < 0.001$).

Table 3 Correlation* between rearing responses and locomotor activity in mice from different generations

Population	n	r_s	P
P ₁ (SRH)	20	0.5564	<0.01
P ₂ (SRL)	20	0.6703	<0.001
F ₁	30	0.5540	<0.001
F ₂	30	0.7509	<0.001
B ₁	30	0.6987	<0.001
B ₂	30	0.7582	<0.001

*Spearman rank correlation coefficients calculated from raw data.

must be the result of variation in the same genetic factor or factors. The locus which controls rearing frequency and the locus which influences locomotor activity may be identical (pleiotropy) or, perhaps, closely linked (gene block).

This report demonstrates that it is possible to single out by means of selective breeding one locus responsible for differences in a quantitative behavioural variable in mice, the reaction to novelty. Previous investigations with the anticholinergic drugs scopolamine and methylscopolamine provided evidence that the genetic factor acts through a hippocampal cholinergic mechanism which regulates exploratory tendencies¹. The factor may be allelic with the gene *Exa* (for exploratory activity level; identified by using recombinant inbred strains), the action of which can be changed by another locus designated *Sco* (scopolamine-induced modification of exploratory activity¹²). Apart from the isolated gene, other allelic differences that were not maintained during the selection process may also have contributed to the behavioural difference between the ancestral strains.

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The courtship of the male has been described several times^{3–5}: the male orientates to the female and vibrates one or both wings, producing a signal important in species recognition⁶ and in the sexual stimulation of the female^{7–9}. The male extends the proboscis and licks the genitalia of the female, at the same time flexing the abdomen in preparation for attempts at copulation. During this procedure the tarsal segments of the forelimbs, with the associated sex combs, are used to grasp the genital region of the female¹⁰.

Females showing lesbian behaviour clearly demonstrate several of these male behaviours. The female orientates to and follows other females, sometimes engaging in a bout of foreleg tapping³. Head-on 'courtship' occurs with both wings extended, a relatively rare phenomenon even in males (Fig. 1 b). Most of the behaviour is, however, directed to the genitalia of the courted female (Fig. 1 a), where following and wing extension commonly occur with the tarsi of the forelegs used in a behaviour similar to, but far less precise than, the grasping performed by males; in females it might be called groping. It is not yet known whether an acoustic signal is produced during wing extension, but this behaviour seems to be similar to, although of shorter duration than, the wing vibration of males. In lesbian females it often intergrades into the flicking behaviour² of both normal females and males.

Although this behaviour may be performed extremely vigorously, it is broken into discrete bouts of short duration, usually of less than 20 s (mean = 4.68 ± 0.33 s); such bouts may, however, follow one another in quick succession, and on occasions the behaviour becomes effectively continuous. Even in the individuals which show the behaviour most intensively I have not observed proboscis extension and licking of the genitalia; attempted copulation is also absent. A response to lesbian behaviour by sibling virgin females is extrusion of the genitalia; flicking also frequently occurs.

The observations were conducted on pairs of females placed at eclosion in cylindrical plastic cells (30 mm high, 22 mm in diameter), filled to within 1 cm of the top with food medium. Observations were made by repeated scanning of the sample of cells in the evening of days 2 and 3 after eclosion. A female was only classified as of lesbian phenotype when the behaviour had been observed more than once.

Lesbian behaviour has as yet only been found in a series of related 'balanced lethal' strains, designed to maintain the gene *Fs* (2)B (female sterile) in the heterozygous state. In the strain *Fes31*, for example, samples from four different generations gave a mean of $23.7 \pm 4.0\%$ females showing lesbian behaviour. The composition of *Fes31* with respect to chromosome II is:

$$fs\ dp^+ \ Sp/al^2 \ Cy\ (In2LR)\ cn^2 \ L^4 \ sp^2$$

Lesbian behaviour is not genetically dependent on any of these mutations on chromosome II, since the behaviour is easily retained when both chromosomes II are substituted for those of a laboratory strain in which lesbian behaviour has not been observed (Pacific and Novosibirsk strains).

The crossing carried out to transfer the factor(s) responsible for lesbian behaviour to a non-mutant genetical background, rearing in groups rather than single families, has provided the data shown in Table 1. This suggests that *Pacific* is genetically

Table 1 Preliminary genetic data on lesbian phenotype

10 <i>Fes31</i> (♀♀ showing lesbian behaviour) × 10 <i>Pacific</i> ♂♂	Total no.	No. of lesbian females	% Lesbian
F ₁	162	17	10.5
F ₂	331	98	29.6
10 F ₂ (♀♀ from above cross showing lesbian behaviour) × 10 <i>Pacific</i> ♂♂			
F ₁	74	3	4.0
F ₂	72	25	34.7

'Lesbian' phenotype of *Drosophila melanogaster*?

A BEHAVIOURAL phenotype of female *Drosophila melanogaster* has been found which is characterised by the emission of what seems to be a somewhat rudimentary form of male courtship behaviour; it is directed towards other females, and also towards males. 'Lesbian' behaviour has not been reported before, although it may occur in other strains of *D. melanogaster*.

Female *D. melanogaster* do not usually seem to initiate courtships (although see ref. 1 for factors influencing their attractiveness to males), but take a somewhat passive role—unless they are unreceptive, when a repertoire of rejection responses comes into play². The most important of these rejection behaviours is extrusion of the genitalia towards the courting male.

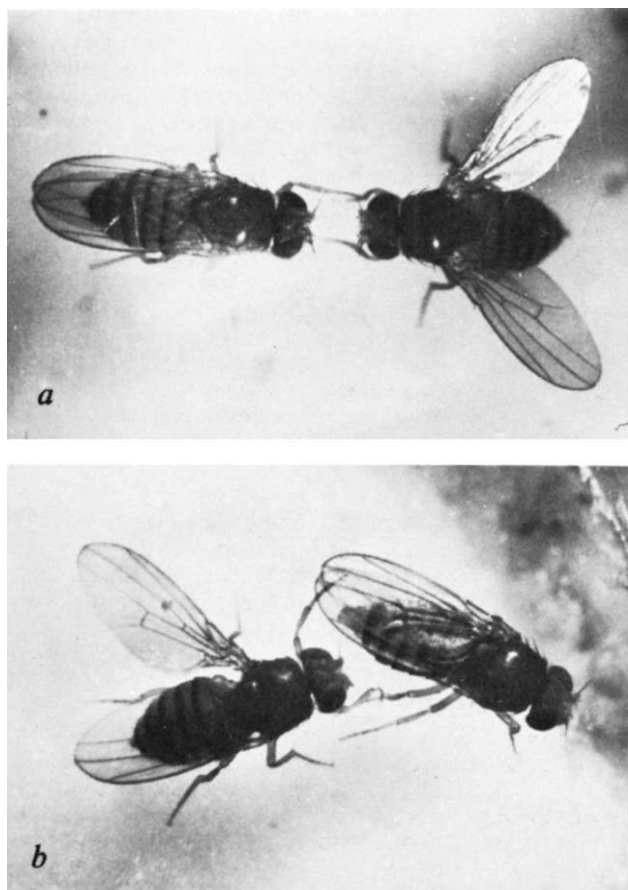


Fig. 1 *a* and *b*, 'Courtship' behaviour of lesbian phenotype females. $\times 12$.

different to *Fes31* with respect to the factor(s) controlling lesbian behaviour, with the *Pacific* allele(s) showing incomplete dominance. Conclusions as to the mode of inheritance of this behaviour must await further genetical and behavioural studies.

Analysis of the data gained from the observations described above shows that lesbian females are found together more frequently than would be expected by assuming a random distribution of such females across the cells (Table 2). In all cases the observed frequencies differ with the same pattern from

those expected. It is thus possible that lesbian females mutually stimulate each other into manifestation of lesbian behaviour. This implies that some potentially lesbian females are not detected by the measuring procedure used and that this is therefore an underestimate of the proportion of such females in the sample.

Although this behavioural phenotype closely resembles certain aspects of male courtship behaviour it cannot yet be definitely asserted that it derives from this system. It may, for example, have evolved to serve a function of 'spacing' or 'aggression' between females, or as a response to the abnormal conditions imposed by the mutations of the balanced lethal system.

Whatever its function and origins may prove to be, lesbian behaviour is an item in the (expanding) repertoire of *D. melanogaster* (see for example the 'homosexual' mutant of Gill¹¹), on which genetical techniques of analysis may be brought to bear. More detailed behavioural and genetical studies are at present under way.

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Entrainment of the pupation and adult activity rhythms during development in the mosquito *Anopheles gambiae*

PREVIOUS work on the initiation of the pupation and flight activity rhythms in mosquitoes¹⁻³, has been interpreted⁴ as showing that there is an ontogenetic distinction between the 'clocks' controlling development and adult behaviour. Results with *Aedes taeniorhynchus*^{1,2} suggest that the developmental clock stops after controlling the time of pupation and that the adult behavioural clock does not start until some time after emergence. In *Culex pipiens pallens*³ the adult clock seems to start just before adult emergence. By contrast, the results reported here indicate that in the mosquito *Anopheles (Cellia) gambiae* Giles, the flight activity rhythm can be entrained during development at least as early as the second larval stage. The same Zeitgeber also sets the phase of the pupation rhythm.

The insects used were *A. gambiae* species A from a culture originating in Pala, Upper Volta. They were reared in LD 12:12 (alternating 12 h light: 12 h dark; 300 lx) and then placed in DD (constant dark) either at the normal time of light-off or after prolonging the light period. Similar experiments with the adults have shown that prolonging the light period resets the flight activity rhythm⁵. In the present experiments 20-40 adults emerged into a muslin cage (300 mm cube) and the total flight activity was monitored using the acoustic actograph technique^{6,7}; as in previous experi-

Table 2 Statistical analysis of the observed permutations of lesbian / non-lesbian individuals in the observation cells

No. of lesbian flies per cell	Expected	No. of cells containing permutations shown	Observed values	Statistical test
Strain				
<i>Fes31</i>				
2	5.76	12		
1	30.12	18		
0	39.76	46		$\chi^2 = 12.59, P < 0.001$
<i>s/fs</i> (sterile females)				
2	6.20	10		
1	25.80	18		
0	30.02	34		$\chi^2 = 4.82, P < 0.10$
<i>Fes30</i> $\times$ <i>Novosibirsk</i> , F_4				
2	4.79	8		
1	16.31	10		
0	13.89	17		$\chi^2 = 5.25, P < 0.10$
<i>Fes30</i> $\times$ <i>Novosibirsk</i> , F_4				
2	9.72	21		
1	33.10	16		
0	28.18	34		$\chi^2 = 23.18, P < 0.001$

ments, a score of 1 was given for any flight activity in any minute. The temperature was maintained at 27 °C.

In the first series of experiments, the change to DD was made during the pupal stage. In an LD 12:12 regime, pupation takes place mainly in the last 6–9 h of the light period, reaching a peak 3–4 h before the end of the light phase. In each experiment, 30–40 pupae, which had pupated between 3.5 and 4.5 h before the normal end of the light phase, were given the final 'light-off' either at the normal time (in this case about 4 h after pupation) or after an extra 12 h of light. In all experiments there was a clear cycle of adult activity which was in phase with the time of the final light-off; the period of the cycle was about 23 h as in previous flight activity experiments⁵. During this series of experiments, adult emergence was monitored under infrared illumination (continued throughout the dark period) using an infrared sensitive TV camera (Link 101S)⁸. The adults emerged 26–30 h after pupation and the time of emergence did not seem to be affected by the difference in the time of light-off. This is consistent with Nayar's observation¹ that the time of emergence in *Ae. taeniorhynchus* is dependent on the pupation rhythm, the duration of the pupal stage being determined mainly by temperature.

In a second series of experiments, the change to DD was made during the larval stage. Figure 1 shows the flight activity of the adults when the change was made during the fourth (final) larval stage and also the timing of pupation in a parallel series of experiments in which mixtures of third and fourth stage larvae were put into constant dim red light. In both series of experiments the change was made either at the normal time of light-off or after prolonging the final light period for 9 h. In the pupation experiments, the pupae were collected from the larval bowls at hourly intervals. In DD (dim red light) there is a cycle of pupation with a period of just under 24 h. Prolonging the final light period does not affect the timing of the first peak, but resetting is complete by the third peak, which seems to correspond to the second peak after light-off at the normal time. Between these two peaks is another smaller peak; this may consist of those individuals which were sufficiently advanced to pupate towards the end of the first reset 'gate'. These results suggest that, if the clock is reset immediately by the extra light, the contribution of the clock to the timing of pupation must take place 24–30 h before pupation actually occurs.

The rhythm of flight activity does not seem to be affected by the actual time of pupation. The flight activity (in DD) of adults derived from pupae selected from these pupation experiments shows that the rhythm is reset by prolonging the final light period, even in individuals which pupate in

Fig. 1 Pupation and subsequent adult flight activity after a change to DD late in the larval stage, either at the normal time of light-off or 9 h later. ●, Median of pupation peak.

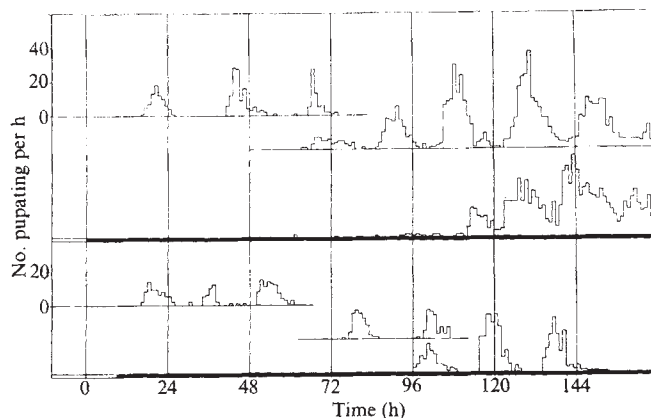
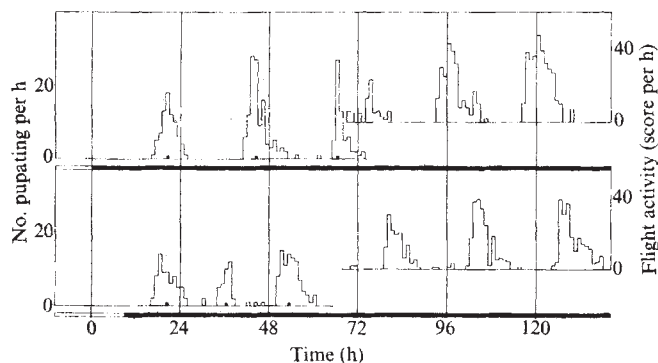


Fig. 2 Timing of pupation when the change to DD was made with batches of larvae of different ages.

the first peak, in which the time of pupation has been unaffected by the extra light.

Experiments in which the change to DD was made at an increasingly early stage (Fig. 2), indicate that the period of the rhythm controlling pupation remains constant at about 24 h for four cycles and then decreases to 16–20 h. This change in period also occurs when the rhythm is reset by prolonging the final light period. Figure 3 shows the flight activity of mosquitoes which pupated in the peak approximately 90 h after light-off, when the change to DD was made with a mixture of second and third stage larvae. It can be seen that the activity is still cyclical, with a period of about 23 h, and that the phase is determined by the time of light-off. Despite the change in period of the pupation rhythm, mosquitoes which pupated 132 and 144 h after light-off still showed a clear cycle of flight activity, which, in period and phase, was completely consistent with that in Fig. 3.

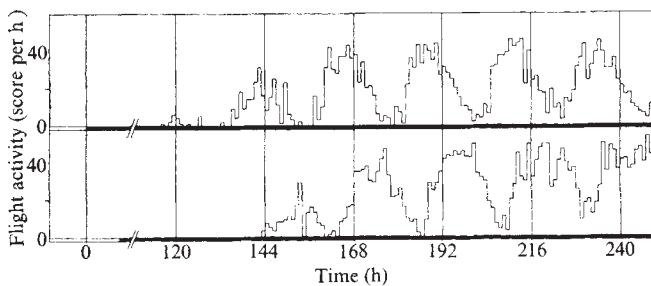


Fig. 3 Flight activity of mosquitoes which pupated approximately 90 h after light-off.

Thus, there seems to be considerable overlap between the 'clocks' controlling the timing of pupation and adult activity and this is surprising in view of findings with other mosquitoes^{2,3}. In *Drosophila*⁹ the evidence implies that the same clock may 'gate' eclosion and control adult behaviour, persisting through development and metamorphosis. By contrast, in *A. gambiae*, the apparent acceleration of the pupation rhythm, the persistence and stability of the flight activity rhythm and the altering phase relation between the two rhythms suggest that we are dealing with two distinct timing processes. If this is so, the function of the early occurrence of the flight-activity rhythm during development remains a puzzle. Possibly it may play some part in the control of larval or pupal activity.

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Chemotaxis of the spermatozoa of *Ciona intestinalis*

CHEMOTAXIS of animal sperm, long thought not to occur¹⁻⁴, was first proved in the marine coelenterate *Campanularia*⁵, and since then has been observed in other hydroids^{6,8}. The species-specificities and cross reactions between sperm and reproductive structures of these species and genera have been described⁶, and some of the attractants have been isolated and their chemical properties reported⁷. In spite of circumstantial evidence for sperm chemotaxis in other phyla^{13,14} the sperm behaviour leading to aggregation has not been described. I now report evidence of sperm chemotaxis to egg water and egg extracts (see Table 1 for details) of *Ciona intestinalis* (Protochordata: Urochordata), a primitive chordate. These results imply that sperm chemotaxis either has evolved independently in groups widely separate on the phylogenetic scale or is widespread in the animal kingdom.

Minganti¹⁰ reported species-specificity of the sperm activation and agglutination responses to egg water in five species of Mediterranean tunicates, including *Ciona*. My own observations of *Ciona* sperm in the vicinity of unfertilised eggs indicated that the eggs, or a preparation of egg water (Table 1), activated nearby non-motile spermatozoa^{10,11} and seemed to induce turning movements¹¹. To investigate whether these turns were chemotactic, *Ciona* gametes were obtained by dissection or by shedding the animals¹² and the methods of observation and photographic techniques previously used on hydroid sperm^{5,9} were applied.

Figure 1a shows a typical preparation of *Ciona* sperm swimming against an albumin-coated glass microscope slide under dark field illumination. The cells were describing circles of either 200-250 nm or 100-120 nm diameter. Some cells switched from one diameter to another and often made short, straight deviations shifting the circle to a new position (not shown). In fresh suspensions the diameter of the circle was much larger and some cells followed long, straight paths, possibly because they had not yet made proper contact with the slide surface. As the preparation aged, the straight trails were seen less often or not at all and the diameter of the circles decreased to about 100 nm. The tight circling behaviour sometimes led to the cell leaving the surface and swimming up into the medium.

When seawater was injected from a pipette at the lower left as shown in Fig. 1b, sperm behaviour was basically unaltered. There was no sign of rheotaxis during the 1-2-s injection. When *Ciona* egg extract or egg water was injected (Fig. 1c-f) the cells aggregated rapidly at the pipette tip, sometimes even if no material was injected. After the pipette was removed the aggregate remained but tended to broaden with time. Figure 1f shows that the cells in the aggregate remained motile and did not agglutinate, though some stuck to the slide surface, particularly where the pipette had scraped away the albumin coating.

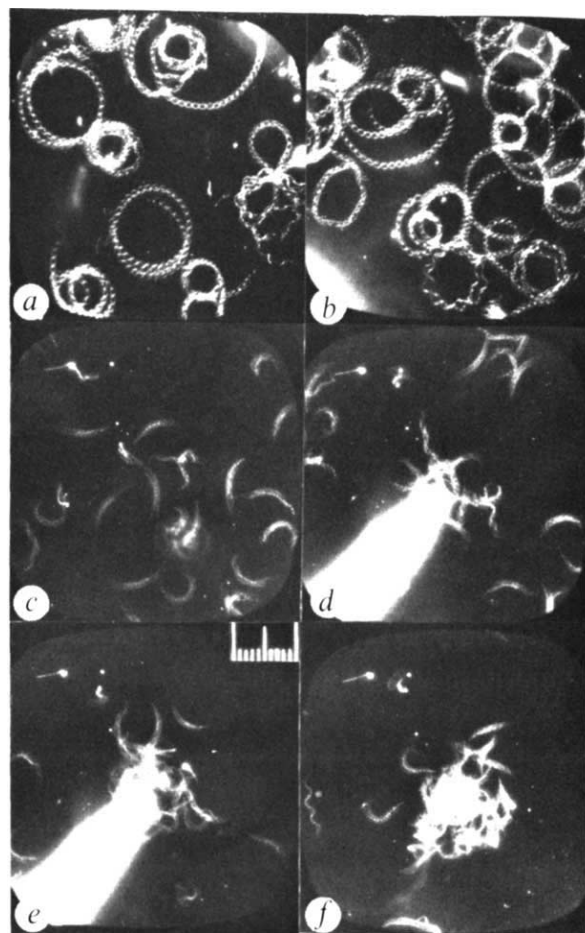


Fig. 1 Behaviour of *Ciona* sperm in the presence of seawater and egg extract injections. *a*, *Ciona* sperm swimming against a glass surface with a beat frequency of 40 Hz. Exposure was for 4 s and flash frequency was 20 Hz. *b*, Similar preparation with a pipette injecting seawater from the lower left, just off the field of view. Exposure was for 2 s and flash frequency was 20 Hz. *c*, Same as (*a*), 0.33 s exposure and flash frequency approximately 40 Hz. *d-f*, Same preparation as (*c*) with a pipette injecting *Ciona* egg extract (*d*, *e*) followed by its removal (*f*). Times between frames are: *c-d*, 2 s; *d-e-f*, 1 s each. The smallest scale division is 10 μ m.

To observe the details of sperm behaviour as they entered the aggregation area, individual cells circling on the slide surface were exposed briefly to egg extract released from a nearby micropipette. Figure 2a and c shows the situation before the attractant reached the sperm and Fig. 2b and d shows sperm behaviour during the subsequent few seconds in two typical experiments. When the sperm perceived the attractant they stopped circling and moved rapidly toward the tip of the pipette. If the paths were not straight toward the tip, the cells reoriented by looping (Fig. 2b), by making a second direct turn (Fig. 2d) or by making minor adjustments in the path (Fig. 2b). These behaviours were not seen if seawater was injected in the same situation.

Not all the sperm responded to the attractant, the proportion of non-responders increasing with the age of the preparation. A flagellar beat frequency decrease from 38-41 Hz to 32-35 Hz was associated with loss of responsiveness. In contrast to *Tubularia* sperm⁹, it has been difficult to identify a single characteristic flagellar response associated with turning. Furthermore, preliminary measurements indicate no marked increase in swimming velocity as the sperm moved towards the source of attractant in spite of small adjustments of beat frequency like that shown in Fig. 2d. This latter observation may, however, indicate merely that the sperm were already swimming at the maximum velocity possible.

Table 1 Specificity of urochordate sperm chemotaxis

Sperm type	<i>Ciona</i>	<i>Styela plicata</i>	<i>Styela clava</i>	<i>Tubularia</i> (Hydrozoa)
Egg extract (titre)				
<i>Ciona</i> (9)	+ (>100)	— (10)	— (4)	— (5)
<i>S. plicata</i> (4)	— (10)	+ (8)	NT	— (2)
<i>S. clava</i> (0)	— (4)	NT	— (4)	NT
<i>Tubularia</i> (12)	— (5)	— (2)	— (2)	+ (>100)

Numbers in parentheses in columns 2–5 indicate numbers of trials. +, Aggregation of motile sperm; NT, not tested. Egg water was prepared by placing several thousand prewashed eggs into 5 ml of seawater and leaving the preparation to stand for 3–5 h at room temperature. The medium was then drawn off the eggs and tested against fresh sperm. Eggs treated in this manner are fertilisable and develop into normal tadpoles. Egg extract was prepared by removing the seawater from several thousand eggs and replacing it with a small volume of 100% ethanol. After a suitable extraction period (30 min at room temperature) the alcohol was drawn off, evaporated to dryness and the residue was diluted into seawater for testing. One hundred *Ciona* eggs, extracted in 50 μ l of ethanol, produced an active seawater solution which could be half-serially diluted eight or nine times before the attracting activity was lost (titre = 8–9). Five times this number of eggs of either species of *Styela*, extracted in the same manner, gave solutions with no activity against *Ciona* sperm. Follicle cells were isolated by passing eggs through a Pasteur pipette several times in calcium–magnesium-free seawater. The eggs were removed by low speed centrifugation (500 r.p.m.) and the follicle cells were collected at 2,000 r.p.m.

Although attractant activity can be obtained by alcoholic extraction of several tissues of adult *Ciona* (particularly those associated with the ovotestis) eggs consistently produced the highest titres. The distribution of attractant activity in adult tissues remains to be determined. Extracts of tissues from immature *Ciona* were inactive. Extracts of isolated outer egg follicle cells were very active and the eggs from which they had been removed remained active (see Table 1 for method). Since all the follicle cells could not be removed from the egg chorions, and since both egg and follicle cell preparations contained a clear, viscous mucus as a persistent contaminant, the exact source of the attractant remains in doubt.

The species-specificity of the response has been partially determined. Alcohol extracts of eggs of the tunicate *Styela plicata* obtained by light-induced shedding (C. Lambert, personal communication), had no effect on *Ciona* sperm, but they did attract sperm of *S. plicata*. Extracts of *Ciona* eggs of rather high titres (Table 1) had no effect on the sperm of *S. plicata*. No chemotaxis has been observed in the sperm of *Styela clava* toward any of the egg extracts used, even those prepared from eggs of this species. There is no cross reactivity between the sperm and sperm attractants of

Tubularia and *Ciona* (Table 1). Curiously, although *Ciona* is known to be largely self-sterile¹³, egg extracts prepared from any one animal will attract sperm obtained from that same animal.

Observations that chemotaxis of the sperm of the hydroid *Hydractinia echinata* occurs to eggs of that species¹¹ have confirmed directly the ability of spawned eggs, free of complex outer investments, to attract spermatozoa species-specifically. *Ciona* also sheds its eggs before fertilisation, and though the egg bears a complex array of investing cells, it nevertheless produces a substance capable of attracting sperm species-specifically. Therefore, arguments^{16,17} that fertilisation of animal eggs in an aqueous medium occurs as the result of chance encounters with sperm may have to be re-evaluated. A possible advantage of sperm chemotaxis to spawned eggs comes from the effective increase in volume of the egg as a target for the sperm. Its further role in animal fertilisation remains to be determined.

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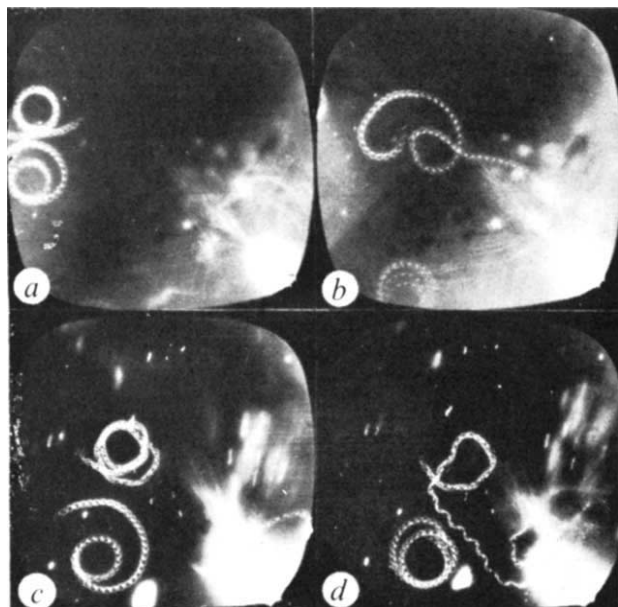
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Fig. 2 Sperm attraction by *Ciona* egg extract. *a* and *c*, Two cells circling on a glass slide close to a pipette injecting egg extract (beat frequency approximately 40 Hz). Exposure: *a*, 2.5 s; *c*, 1.5 s; flash frequency was 17 Hz. *b* and *d*, Less than 1 s later. Exposure: *b*, 3 s; *d*, 2.25 s; flash frequency was 17 Hz. *b*, Response of the lower of the two cells in (*a*). The change in beat frequency for the upper cell in (*d*) as it approached the pipette was an increase of about 1.5 Hz. Same scale as in Fig. 1.



Different drugs arrest cells at a number of distinct stages in G₂

SEVERAL physical and chemical agents can induce susceptible mammalian cell populations to accumulate in the G₂ phase of the cell cycle^{1–3}. The question arises whether cells treated with different agents all accumulate at a single 'restriction' stage within G₂ or, alternatively, stop at a variety of stages.

Techniques of cell-cycle analysis can show the precise

stage at which cycle progression capacity is lost in cells treated with inhibitory substances. If a drug is added to an exponentially growing culture, the time between addition and change in growth rate determines the time in the cell cycle preceding division—the M/G₁ boundary—at which the agent inhibited progression⁹. Cells at a stage beyond this temporal marker at the time of addition continue through the cycle and divide, while those at earlier stages are prevented from progressing at the normal rate. The time is calculated back from M/G₁, since division is the parameter measured.

To determine the precise stage in G₂ that selected chemotherapeutic agents stopped cell-cycle progression, I measured the concentration of cells in exponentially growing cultures of CHO Chinese hamster cells at different times before and after addition of drugs. Figure 1 shows data obtained by treating cells with various concentrations of neocarzinostatin, a compound effective against human leukaemias¹⁰. Although the exact mode of action for this drug is unknown, neocarzinostatin apparently interacts with the DNA, causing a change in DNA structure¹¹. The cells continued dividing at the exponential growth rate (culture doubling time of 16.5 h) for 60 min after addition of the drug; thereafter, in cultures receiving large concentrations of the drug, there was no further increase in cell number, and there was even an indication of a slight loss in the number of cells. With lower concentrations, where the cell cycle was incompletely inhibited, the rate of increase in cell number was decreased, commencing 60 min after addition of drug. Although the rate of progression for affected cells was different at the three drug concentrations, a value of 60 min was obtained in all three cultures for the time interval between addition of the drug and change in the rate of division. Therefore, with a wide (20-fold) range of drug concentrations, the terminal point of action of neocarzinostatin within G₂ appeared to be independent of concentration. (A similar effect was obtained with the other agents studied.) Continued incubation of these cultures for 36 h, in the presence or absence of drug (that is, cells resuspended in drug-free medium after treatment with neo-

Fig. 1 Determination of the terminal point of action of neocarzinostatin in line CHO Chinese hamster cells. A culture of exponentially growing CHO cells in F-10 medium, supplemented with 10% calf serum and 5% foetal calf serum, was split into four subcultures. At time zero, one culture received no drug and served as the control (○). Neocarzinostatin was added to the other cultures, yielding final concentrations of 50 $\mu\text{g ml}^{-1}$ (▲), 400 $\mu\text{g ml}^{-1}$ (◆), or 1,000 $\mu\text{g ml}^{-1}$ (■). Cells were counted at hourly intervals with an electronic cell counter.

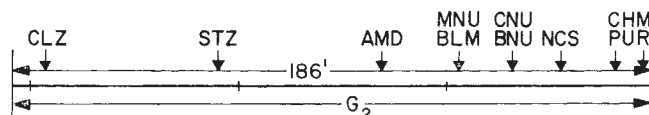
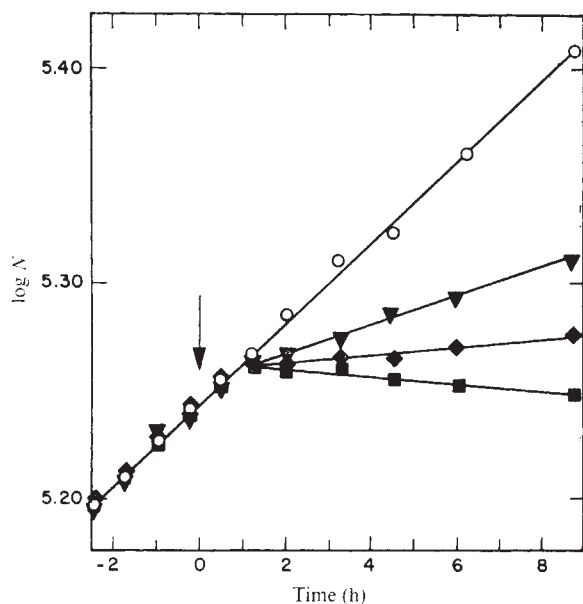


Fig. 2 Drug-specific arrest of progression at different stages within the G₂ phase of the cell cycle in asynchronous cultures of CHO cells. The terminal point of action was determined as in Fig. 1 for the following agents: CLZ, chlorozotocin, NSC No. 178248; STZ, streptozotocin, NSC No. 85998; AMD, actinomycin D, NSC No. 3053; MNU, 1-*trans*-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Me-CCNU), NSC No. 95441; BLM, bleomycin, NSC No. 125066; CNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), NSC No. 79037; BNU, 1,3-*bis*-(2-chloroethyl)-1-nitrosourea (BCNU), NSC No. 409962; NCS, neocarzinostatin, NSC No. 69856; CHM, cycloheximide, NSC No. 185; and PUR, puromycin, NSC No. 3055. Drugs were obtained through Drug Research and Development, Division of Cancer Treatment, National Cancer Institute. The drug solutions were prepared immediately before use. The terminal point of action for each agent was determined in multiple cultures using 20–80-fold concentration ranges for each drug.

carzinostatin for 1 h), resulted in populations comprised almost entirely of cells in G₂, based on measurements of population DNA content obtained with the Los Alamos flow microfluorometer⁸. Thus, there was a net increase in cells in G₂, indicating that the cells initially in late G₂, M, G₁ or S could reach G₂, but no further. Some irreversible change occurred in response to treatment with neocarzinostatin that predisposed the cells to stop in G₂, regardless of the stage of the cell cycle in which they resided while exposed to the drug.

In similar fashion, the terminal point of action was determined for various drugs which cause cells to accumulate in G₂, as shown in Fig. 2^{8,12,13}. The agents tested caused the cells to stop in different portions of G₂. Thus, at least in so far as the agents studied are concerned, cells do not stop at a single stage within G₂. Rather, the data seem to agree well with the model proposed by Mitchison¹⁴ for multiple transition points within G₂.

With the exception of the protein synthesis inhibitors (puromycin and cycloheximide), all agents tested caused at least a partial enrichment in the fraction of cells in G₂, similar to the effects of neocarzinostatin. Although accumulation of cells in G₂ was possible with these agents, the effects on progression capacity were not readily reversible, so that none of these agents seem to be useful in preparing synchronised populations for study of late interphase events.

I have not devised a testable model to encompass all the observations reported here. The well documented arrest of mammalian cells in G₂ after X-irradiation, however, shares many features with the results presented here^{1–4}. Perhaps the consequences to the genome of X-irradiation and treatment with these agents are similar in mammalian cells^{15,16}, although the actual biochemical mechanisms may be different.

I should like to hypothesise that a surveillance mechanism operates throughout G₂ to eliminate from the proliferative mode cells with altered DNA. Such a surveillance mechanism would examine cells at multiple stages within G₂ to ensure that either the damaged DNA was repaired normally, in which case a cell would be returned to cycle, or an unrepaired cell would be converted to a non-viable state, even though several abortive rounds of division might occur before cell death. (From an evolutionary standpoint, conversion to a nonviable state would limit the dissemination of grossly deranged mutant cells and would decrease the frequency of survival of the damaged cell in competing with normal cells for essential nutrients.) Thus, in this model, G₂ would represent a crucial period during which an essential decision is made concerning further cellular proliferation. This model does not exclude the possibility that

additional proliferation decisions could be made in other portions of the cell cycle.

The hypothetical surveillance mechanism is not foolproof, since viable mutants have been isolated from mammalian cell lines^{17,18}. Of course, a totally foolproof mechanism would preclude even evolutionary changes. I feel that drugs such as we used may provide valuable probes for future studies of proliferation control mechanisms.

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Table 1 Properties of mouse fibroblast lines used

	3T3	Py3T3
Generation time (h)	23.5 ± 0.1	29.1 ± 0.6
Saturation density (cells cm ⁻²)	6.4 × 10 ⁴	86.0 × 10 ⁴
Transplantability	Negative	Positive
Agglutinability (HMA)		
RIS (150 mM)	650	75
LIS (< 50 mM)	75	75

Transplantability was tested by injecting 20 Swiss albino mice (immunosuppressed using 450 rad, whole body) with 10⁷ viable cells from logarithmic-phase cultures. Py3T3 cells caused progressive tumour growth in 17 out of 20 mice, compared with 0 out of 20 when injected with 3T3 cells. All agglutination assays used 5 × 10⁵ cells per ml, collected from log-phase cultures, rinsed twice with phosphate-buffered saline (PBS) containing 5 mM EDTA, followed by suspension in PBS containing 10 mM CaCl₂. Con A was added using constant volumes, 0.5 ml cells and 0.5 ml con A, incubated for 30 min at 37 °C, and 125 Hz min⁻¹. HMA is defined as the concentration of con A (μg ml⁻¹) required to agglutinate 75% of the cells. LIS condition was a 30 min incubation before addition of con A. Control agglutination (no con A) did not exceed 10%. Percentage of cells agglutinated was calculated by multiplying average clump size by number of clumps compared with free cells.

enhanced agglutination was observed once the ionic strength was below 50 mM (Fig. 2).

The following properties of this induced agglutinability were demonstrated in parallel experiments: (a) enhanced agglutination was only observed when con A was present with the cells at LIS, that is, incubation at LIS without con A, followed by addition of con A and assay at RIS did not enhance agglutination; (b) once cells were incubated with con A at LIS, agglutinability was enhanced when assayed either at LIS or RIS and (c) the LIS effect was not altered by removal of sodium, potassium or chloride ions (shown by holding the ionic strength constant using other monovalent salts).

Table 2 summarises the nature of the LIS effect with an attached cell preparation using human erythrocytes⁶. The LIS effect was temperature-sensitive, con A-specific, blocked by prior fixation of the cell membrane and not affected by inhibitors of metabolic energy.

These data demonstrate the importance of the ionic environment at the cell surface in determining agglutinability. The enhanced agglutination of 3T3 cells at LIS was phenomenologically identical to the agglutination of Py3T3 cells at RIS by the following criteria: (a) the LIS effect was abrogated using an inhibitor of con A binding; (b) the effect was temperature-sensitive and unaffected by inhibitors of metabolic energy; (c) the HMA was the same value at LIS

Induced agglutinability of 3T3 mouse fibroblasts

CONCAVALIN A (con A) preferentially agglutinates polyoma virus-transformed 3T3 cells (Py3T3) compared with 3T3 cells¹. Brief proteolytic treatment of 3T3 cells results in agglutinability comparable with that of Py3T3 cells, and this is consistent with the suggestion that constraints at the 3T3 cell surface prevent migration and clustering of con A receptors¹⁻³. We now report that 3T3 cells are as equally agglutinable as Py3T3 cells after a brief exposure to low ionic strength conditions (LIS, isosmotic). The data suggest that the mobility of con A receptors is altered by electrostatic interactions at the cell surface.

Py3T3 and 3T3 cells were cultured on plastic substrate at 37 °C, pH 7.0, in Dulbecco's modification of Eagle's MEM, supplemented with 10% foetal calf serum and antibiotics (Grand Island Biological Co.). Preliminary experiments showed that agglutination by con A was a function of time, pH, con A concentration, cell density and oscillations per min for the reaction vessels⁴. Osmolarity was maintained at 340 mosmol for all assays using D-mannitol, which does not inhibit con A binding⁵. Hapten-inhibition with α-methyl-D-mannoside was used to confirm con A specificity. The con A was grade III, twice crystallised (Sigma).

Some properties of Py3T3 and 3T3 cells, determined in this laboratory are shown in Table 1. Half-maximal agglutination (HMA, defined as the concentration of con A required to agglutinate 75% of the cells) was 650 μg ml⁻¹ for 3T3 cells compared with 75 μg ml⁻¹ for Py3T3, when assayed at regular ionic strength (RIS, 150 mM). Prior incubation of 3T3 cells for 30 min at LIS in the presence of con A increased the agglutinability considerably (Fig. 1). The HMA was shifted to 75 μg ml⁻¹, and the concentration dependence was similar to that observed for Py3T3 cells at RIS. This

Fig. 1 The concentration dependence of agglutination with con A is shown for Py3T3 cells at RIS (150 mM) (---); 3T3 cells at RIS (●); and 3T3 cells at LIS (< 50 mM) (○). Agglutination assays performed as described in Table 1 and values represent the means of triplicate determinations.

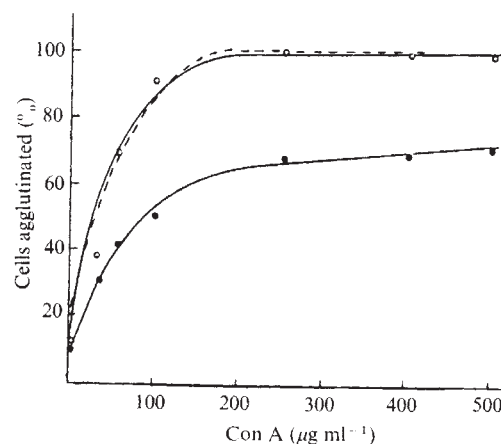


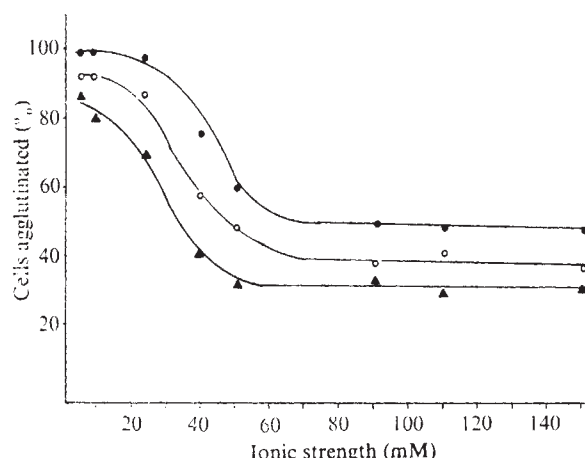
Table 2 Effect of various treatments on enhanced agglutinability of 3T3 cells at LIS

Treatment	Effect on agglutination
37 °C	Enhanced agglutination
25 °C	Enhanced agglutination
4 °C	No agglutination
Formalin (10%, 30 min)	No agglutination
Glutaraldehyde (2.5%, 30 min)	No agglutination
2,4-Dinitrophenol (10 mM)	Enhanced agglutination
Sodium azide (10 mM)	Enhanced agglutination
Potassium cyanide (10 mM)	Enhanced agglutination
α -Methyl-D-mannoside (50 mM)	No agglutination

Cultures were rinsed with PBS, pH 7.2, and incubated at LIS with 340 mM D-mannitol, buffered to pH 7.0 with 10^{-5} M phosphate. Haemadsorption assay used human type O erythrocytes¹² layered over the attached cells at a 1% concentration for 30 min, then removed by gentle rinsing with PBS. Results shown above used monolayer 3T3 cells treated with con A ($50 \mu\text{g ml}^{-1}$) after the indicated cell treatment. Enhanced agglutination indicates heavy clusters (> 10 per cell) of erythrocytes attached to 3T3 cells; no agglutination indicates background adsorption (0–3 per cell). Similar results were obtained using erythrocytes treated with con A, then assayed against untreated 3T3 cells.

for 3T3 cells as observed for Py3T3 cells at RIS and had the same concentration dependence, and (d) it was not necessary to digest the cell membrane with proteolytic enzymes to enhance agglutinability.

The above observations are consistent with the following concept of electrostatic interactions at the cell surface on addition of con A. Lipid bilayers are sensitive to the ionic environment^{7,8}, and binding con A results in an increased surface charge density⁹. Sufficient instability of lipid may require the reorganisation of lipid structure (to maximise order and decrease surface charge density) resulting in the

**Fig. 2** Agglutination of 3T3 cells as a function of ionic strength. Con A at $200 \mu\text{g ml}^{-1}$ (●), $100 \mu\text{g ml}^{-1}$ (○), and $50 \mu\text{g ml}^{-1}$ (▲).

migration and clustering of con A receptors. Clustered binding sites could be stabilised due to cross-linking by con A. The threshold for lipid instability is considered low for 3T3 cells compared with Py3T3 cells since the membrane lipid is inherently more fluid in the transformed cell¹⁰. A low threshold for 3T3 cells is obtained by incubation at LIS, which increases the surface charge density¹¹, and addition of con A causes threshold instability resulting in the clustering of con A receptors. Although other possibilities exist, the concept of a threshold electrostatic condition for the instability of membrane lipid is consistent with the inherent

differences in agglutinability of Py3T3 and 3T3 cells, and enhanced agglutination at LIS.

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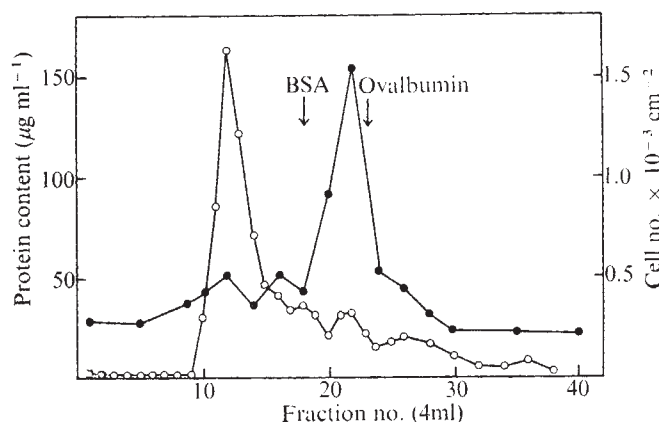
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Growth-enhancing protein obtained from cell surface of cultured fibroblasts

NORMAL animal cells do not grow at extremely low cell density under ordinary culture conditions^{1,2}. This property of population dependence on cell proliferation has long been a crucial disadvantage for establishing clonal cultures of animal cells. Feeder layers or conditioned media are therefore used to sustain the growth of single cells in culture. The growth-enhancing material in conditioned media, the conditioning factor, is thought to be a large aggregate of macromolecules of pericellular environment². This assumption was confirmed by the findings that cultured chick embryo fibroblasts laid down a microexudate carpet on the surface of Petri dishes and that this carpet exerted a growth-enhancing activity on sparsely seeded fibroblasts^{3,4}. Although the microexudate was thought to be composed mainly of glycoproteins, its exact chemical nature has not been known³⁻⁵. Here we report the solubilisation and purification of a growth-enhancing protein from the microexudate of cultured chick embryo fibroblasts.

Kondo and Sakai⁶ extracted a cell aggregation factor by dissociating sea urchin embryos with 1 M urea–5 mM EDTA solution and showed that this factor was precipitated in the presence of calcium ions. This method was used here for the extraction and purification of the exudate of cultured fibro-

Fig. 1 Purification of the growth-enhancing protein on a column of Sephadex G-200. Calcium precipitate obtained from 100 Petri dishes (10 mg protein) was dissolved in 1 ml of 6 M urea–10 mM EDTA at pH 8.0, stored at 4 °C for 2 weeks and then loaded on a column of Sephadex G-200 (1.5 × 75 cm). Elution was carried out with 10 mM Tris–HCl buffer containing 0.1 mM EDTA at pH 7.5 and fractions of 4 ml were collected. Aliquots of 0.2 ml were used to determine the growth-enhancing activity on sparse cell cultures in the same way as described in Table 1. The arrows indicate the positions of bovine serum albumin and ovalbumin used as the standards.



blasts. Monolayer cultures of chick embryo cells were prepared from 9 or 10-d-old chick embryos in Eagle's MEM supplemented with 5% foetal bovine serum. Cells were cultivated for 3–4 d until they reached confluence in 10-cm Petri dishes. Media was discarded, cell layers were washed twice with phosphate buffered saline containing 0.1 mM EDTA and incubated with 5 ml of 10 mM Tris buffer containing 1 M urea and 5 mM EDTA (pH 8.3) at 37 °C for 30 min or at room temperature for 2 h. The cells became round and tended to become detached from dishes, and they were then dissociated from the dishes by gentle pipetting. More than 99% of cells were viable as judged by staining with trypan blue. After removal of cells and debris, the extract from 100 dishes was concentrated to 50 ml with a Diaflo PM-10 membrane. The crude extract showed strong growth-enhancing activity when added to sparsely seeded cell cultures (Table 1, experiment a). The maximum growth-enhancement was obtained by $40 \mu\text{g ml}^{-1}$ of the crude extract, but the extract at higher concentrations was somewhat inhibitory to the cell proliferation. This effect was caused by a macromolecular growth inhibitor separated by subsequent purification (unpublished work). Quantitative assay of the growth-enhancing activity was difficult because the assay depended strictly on the inoculum density and physiological condition of the cells and because of the existence of inhibitory substances. The degree of subsequent purification, however, could be estimated roughly in terms of the doses required for maximum enhancement.

Table 1 Growth-enhancing activity of the crude extract and calcium precipitate on cultured chick embryo fibroblasts

Experiments	Dose (protein content $\mu\text{g ml}^{-1}$)	Stimulation of growth (Cell no. $\times 10^{-3} \text{ cm}^{-2}$)
a, Control		2.0 (± 0.3)
Crude extract	2	7.9 (± 2.3)
	10	15.8 (± 3.1)
	40	22.3 (± 2.4)
	80	9.3 (± 2.0)
b, Control		0.7 (± 0.2)
Calcium precipitate	2	2.6 (± 0.2)
	5	2.9 (± 0.4)
	10	4.6 (± 0.2)
	20	3.7 (± 0.3)
Calcium-supernatant	5	0.8 (± 0.1)
	10	0.8 (± 0.1)
	20	0.5 (± 0.2)

All samples were dialysed against 10 mM Tris-HCl buffer containing 0.1 mM EDTA at pH 7.5 before the determination of the growth-enhancing activity. Aliquots of 0.02 to 0.2 ml of samples were sterilised in 3.5 cm Falcon plastic Petri dishes by brief ultraviolet irradiation (at a distance of 30 cm from a 15 W germicidal lamp for 5 min). Cultivation was started at a density of 500–1,000 cells cm^{-2} in 2 ml of Eagle's MEM containing 5% foetal bovine serum, and the numbers of cells were counted after 7 d. At these low cell densities, the growth rate in the control plates was very low³. All experiments were done in duplicate. Protein content was determined by the method of Lowry *et al.*¹⁰.

The concentrated extract was dialysed against 10 mM Tris-HCl buffer containing 10 mM calcium chloride at pH 7.5. The white precipitate which appeared in a dialysing tube (about 25% of total protein) was collected by centrifugation and dissolved in 6 M urea–10 mM EDTA at pH 8.0. The activity was found only in the calcium-precipitate fraction (Table 1, experiment b). Since this fraction showed maximum enhancement at $10 \mu\text{g ml}^{-1}$, it was concluded that almost all the activity was recovered in the calcium precipitate.

The calcium precipitation was repeated again and the precipitate (about 20% of total protein in the crude extract) was dissolved in 1 ml of 6 M urea–10 mM EDTA at pH 8.0. After storage at 4 °C for two weeks, the materials were fractionated on a column of Sephadex G-200. The growth-enhancing activity was eluted as a single peak, between the positions of bovine serum albumin and ovalbumin (Fig. 2). This peak contained a single protein as shown by polyacrylamide gel electrophoresis

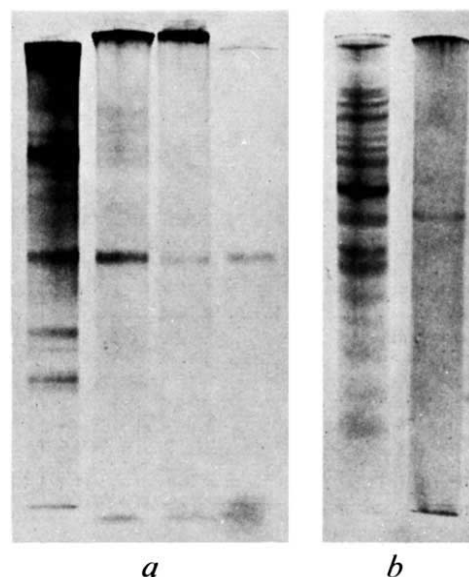


Fig. 2 Patterns of polyacrylamide gel electrophoresis with or without SDS at each purification step. a, Electrophoresis in 7.5% gels without SDS at pH 8.1 (ref. 11). From left to right; crude extract, first calcium precipitate, second calcium precipitate, and purified protein. b, SDS-polyacrylamide gel electrophoresis in 10% gels¹². Left, crude extract; right, purified protein. The molecular weight of the purified protein was estimated to be 48,000 (mean of several experiments) using bovine serum albumin, ovalbumin and lysozyme as the standards.

with or without SDS (Fig. 3), of molecular weight about 48,000. This value agreed with the results of Sephadex G-200 column chromatography (Fig. 2). About 400 μg of purified protein (1.5% of total protein in the crude extract) was obtained from 100 dishes. Prolonged treatment of the calcium precipitate in urea-EDTA solution was necessary to dissociate the active materials into monomers of molecular weight 48,000. When this treatment was reduced, two additional peaks of activity were usually found after fractionation on Sephadex G-200, one corresponding to a molecular weight of about 100,000 and the other at the void volume position. In these procedures, it should be also noted that the abrupt addition of calcium chloride often caused a drop in the pH, resulting in the coprecipitation of inactive materials.

Figure 4 shows the effect of purified protein on the growth rate of cultured fibroblasts seeded at low cell density. The purified protein exerted its maximum growth-enhancing effect at 1 to 2 $\mu\text{g ml}^{-1}$, and the activity was lost completely by treatment with 0.1% trypsin at 37 °C for 30 min. Primary cultures of mouse embryo cells was also stimulated. The activity was retained after brief ultraviolet sterilisation, after treatment with urea or several kinds of detergents, and after freezing-thawing or storage of the solution at 4 °C for at least 2 months. The protein tends to precipitate at a pH lower than 5.0 or in the presence of a small amount of calcium or magnesium ions. Although preliminary experiments indicated that radioactive glucosamine was incorporated into this protein, chemical compositions have not yet been determined because of low yields.

The present investigation has proved that urea-EDTA solution⁶ is also useful for the extraction of cell surface or extracellular materials of higher animals with minimum destruction of cells, because 1 M urea solution is nearly isotonic with the cells. We have isolated the same growth-enhancing protein from chicken embryonic tissues by the direct extraction with 1 M urea–5 mM EDTA solution (Y.Y., unpublished).

As expected previously^{1–4}, the purified protein has properties characteristic of pericellular structural proteins, indicating that this protein is responsible for the growth-enhancing activity of

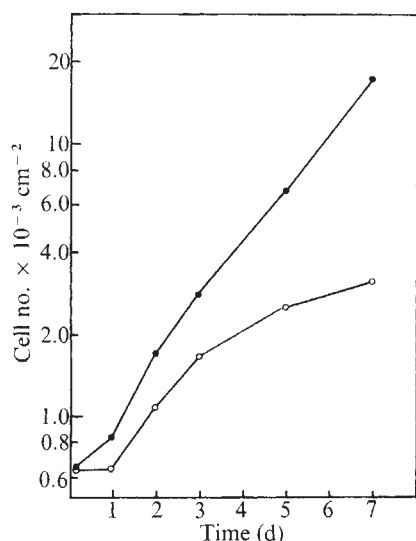


Fig. 3 Effect of purified protein on the growth rate of cultured fibroblasts at low cell density. $\circ$, Control; $\bullet$, $1 \mu\text{g ml}^{-1}$ of the purified protein was added at the start of the cultivation.

conditioned media or feeder layers. Calcium ions may be required for the maintenance of the structural integrity and growth-enhancing activity of the microexudate carpet. In this respect, the carpet protein should be distinguished from humoral growth regulators such as hormones, serum components or fibroblast growth factor⁷. Apparently it resembles closely the "mesenchymal factor" which was partially purified from chick embryonic tissues by Ronzio and Rutter⁸ with respect to its structural protein nature, the molecular weight of the monomer, trypsin-sensitivity, possible contribution of calcium ions for activity, and in its localisation at cell surfaces⁹. These factors may offer a molecular and morphological basis for understanding the contact or short range interactions affecting the proliferation of animal cells, with special reference to the functions of cell surfaces.

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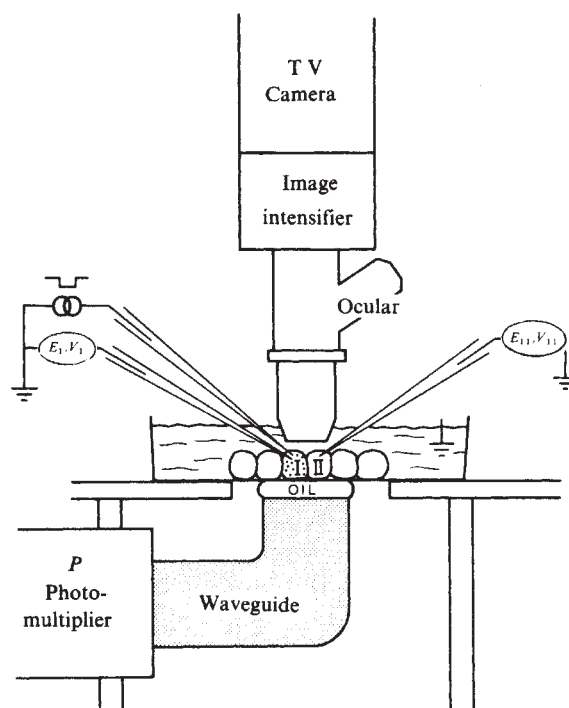
Permeability of cell junction depends on local cytoplasmic calcium activity

MANY kinds of cells are coupled by junctions consisting of membrane channels through which molecules of a certain size range can flow freely from one cell interior to another¹. It has been proposed that the permeability of the junctional channels depends on the concentration of free ionised calcium in cytoplasm ($[\text{Ca}^{2+}]_i$) (ref. 2). This hypothesis is supported by two classes of experiments. In one, the interior of a coupled cell system is allowed to exchange freely with a known $[\text{Ca}^{2+}]$ in the exterior, through a hole in the (non-

junctional) membrane; junctional conductance is reduced (uncoupling) when the $[\text{Ca}^{2+}]_i$ is above $5-8 \times 10^{-5} \text{ M}$ (ref. 3). In the other class, uncoupling ensues when the (closed) cell system is treated with inhibitors of energy metabolism or with Ca^{2+} ionophores³, or on exposure for long periods to Ca, Mg -free medium or to Li medium⁶; in these conditions a rise in $[\text{Ca}^{2+}]_i$ may be expected because of known properties of cellular Ca metabolism^{7,8}. Here, we demonstrate the changes in $[\text{Ca}^{2+}]_i$ together with those in coupling in three of these conditions, using aequorin to display the distribution of Ca^{2+} in the cell. It will be shown that the uncoupling is, indeed, in each case associated with a rise in $[\text{Ca}^{2+}]_i$. Furthermore, by local injection of Ca^{2+} into the cells, it will be shown that uncoupling ensues when the rise in $[\text{Ca}^{2+}]_i$ occurs at the junction, but not when it occurs at other regions in the cell.

Chironomus salivary glands were isolated in physiological medium as described previously⁴, except that the medium contained 12 mM Ca and no Mg . Purified aequorin was injected into one or two adjacent cells. This protein reacts with Ca^{2+} , emitting light. The emission, approximately proportional to $[\text{Ca}^{2+}]^2$ (ref. 9), provides a convenient $[\text{Ca}^{2+}]_i$ indicator¹⁰⁻¹². The aequorin had no apparent adverse effects; membrane potentials and coupling were maintained for several hours. Light emitted inside the cells was guided by a light pipe to a photomultiplier. In addition, a television camera coupled to an image intensifier viewed the cell luminescence through a microscope (Fig. 1). This novel aspect of aequorin technique enabled us to see where the light was emitted inside the cells with a resolution of $5 \mu\text{m}$. Ca buffered with EGTA (to stabilise the $[\text{Ca}^{2+}]$) was pressure-injected into the cell containing the current source, while electrical coupling between this cell and a contiguous cell was measured as shown in Fig. 1. Current pulses, membrane potentials, their displacements, and photocurrent

Fig. 1 Television camera coupled to image intensifier by a light guide views the aequorin luminescence of the cells through microscope (darkfield); luminescence is also measured by photomultiplier. Electrical coupling is measured with the aid of three microelectrodes, by pulsing current (i) between the inside of cell (I) and the outside and measuring the resulting steady-state changes (V) and membrane potentials (E) in I and adjacent cell II.



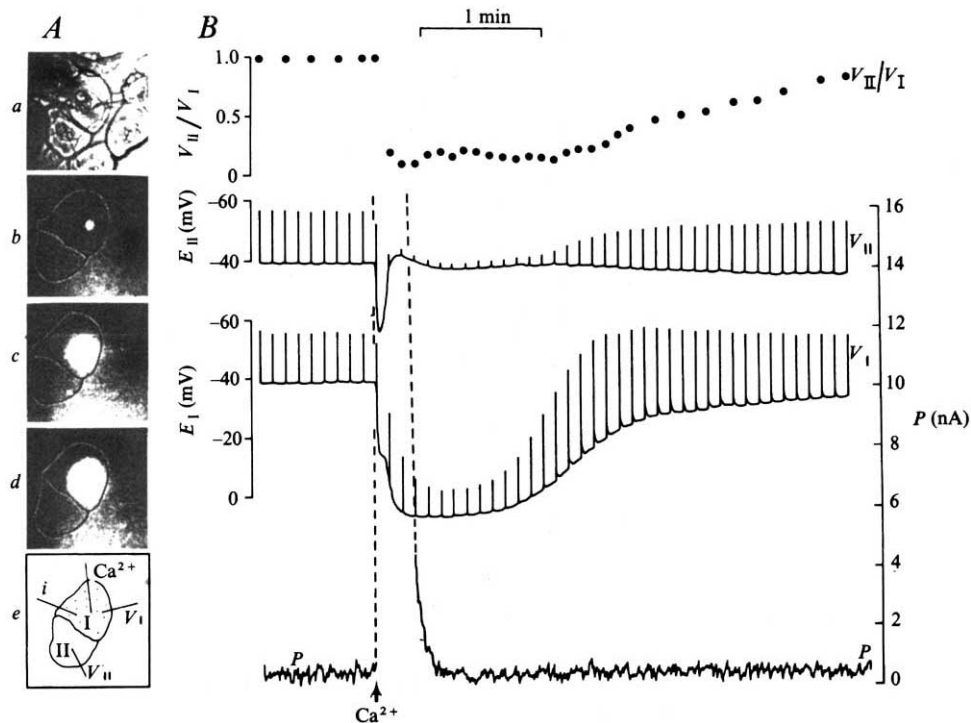


Fig. 2 Ca^{2+} injection. *A*, Dark-field television pictures (*b-d*) of aqaurin luminescence in a cell, produced by three puffs of 5×10^{-5} M free Ca (buffered) of increasing magnitude, delivered to cell centre. The pictures are videotape photographs each taken at the time of maximum luminescence spread. Cell outlines are traced by superposition of brightfield video picture (*a*); cell diameter $\sim 100 \mu\text{m}$. Puffs *b* and *c* do not reach the junctions of the cell and do not affect coupling. Puff *d* reaches one junction and causes transient uncoupling, as shown in *B*: chart records of photomultiplier current P , E_i , E_{ii} , V_i , V_{ii} ($i = 4 \times 10^{-8}$ A); and plot of coupling ratio V_{ii}/V_i . *e*, Cell diagram showing locations of microelectrodes and of Ca injection micropipette; dotted cell preinjected with aqaurin.

were displayed on a chart recorder and on a storage oscilloscope on to which a second television camera was focused. The two camera outputs were displayed together on a monitor and videotaped. Thus, we had continuous and simultaneous information on the electrical parameters and on the relative local Ca^{2+} activities inside the cells.

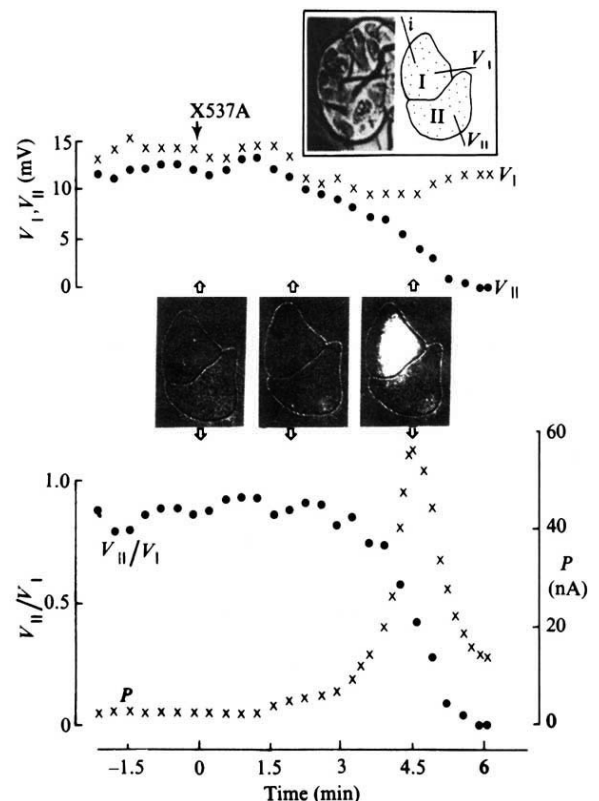
Short single injection pulses of $5-10 \times 10^{-5}$ M free Ca^{2+} into healthy cells produced aqaurin glows that were confined to the immediate vicinity of the tip of the injection pipette (Fig. 2). Evidently the Ca^{2+} is prevented from diffusing freely through the cytoplasm and seems to be sequestered by intracellular elements^{7,13,14}. When such an injection was made into the cell centre (cell radius, $50 \mu\text{m}$), junctional coupling was unaffected. When it was close to a cell junction, the coupling fell (uncoupling), and this effect was confined to the junction at which the local rise in $[\text{Ca}^{2+}]_i$ had occurred. Longer or more frequent injections of this sort, or prolonged Ca^{2+} iontophoresis between electrodes placed in two adjacent cells, produced glows that were diffuse over the entire cell. Then all junctions of the injected cell uncoupled. Presumably the intracellular sequestering capacity was saturated under these conditions. (When mitochondrial sequestering of Ca was blocked by ruthenium red¹⁵, the glow was diffuse even with the shorter injection pulses.) Control injections of 0.15 M KCl did not affect coupling of $[\text{Ca}^{2+}]_i$. Massive KCl injections, however, produced transient and diffuse rise of $[\text{Ca}^{2+}]_i$ associated with transient uncoupling.

Treatment with ionophore A23187 (Lilly, 2×10^{-6} M) or X537A (Hoffman LaRoche, 1×10^{-5} M) led to enhanced Ca influx which was detectable within 1 min as a diffuse glow that increased progressively during the next 10 min. The rise in $[\text{Ca}^{2+}]_i$ was invariably associated with uncoupling, the coupling ratio diminishing progressively with rising $[\text{Ca}^{2+}]_i$ (Fig. 3).

Similar results and equally good correlation between rising $[\text{Ca}^{2+}]_i$ and uncoupling were obtained when the cells were poisoned with sodium cyanide (5×10^{-3} M) in medium containing Ca as well as in Ca-free medium (Fig. 4).

Prolonged exposure to Ca,Mg-free medium led to a rise in $[\text{Ca}^{2+}]_i$. This rise, evidently nurtured by intracellular Ca stores (as Na accumulates inside the cells in Ca-free medium¹⁶, the mitochondria may release Ca^{17}), was asso-

Fig. 3 Ionophore X537A. Exposure to 1×10^{-5} M X537A starts at black arrow and continues throughout the remainder of experiment. Top to bottom: V_i , V_{ii} ($i = 4 \times 10^{-8}$ A); darkfield television pictures of luminescence (open arrows indicate their time correspondence); coupling ratio V_{ii}/V_i ; photocurrent P . Cell I depolarises as P rises above background, reaching zero membrane potential at time 9 min. Peak of luminescence in cell II (not shown) occurred 5 min after peak in I. *Top inset*: bright field television picture and cell diagram; cells I and II contain aqaurin.



ciated with uncoupling. These experiments were particularly instructive, because $[Ca^{2+}]_i$ and coupling fluctuated: rise and fall in $[Ca^{2+}]_i$ were associated, respectively, with fall and rise in coupling.

In cells with good membrane potentials (≥ 40 mV), the uncouplings produced by single short Ca pulses generally were spontaneously reversible (Fig. 2). Presumably the cells rid themselves of the excess Ca by sequestering it into mitochondria and by pumping it out. With more massive injections, or after ionophore treatment or prolonged exposure to Ca, Mg-free medium, the uncouplings did not reverse spontaneously. It was then possible to reverse the uncouplings by injection of Ca-EGTA solution yielding $\leq 10^{-6}$ M free Ca.

In all these conditions, the rise in $[Ca^{2+}]_i$ was accompanied by depolarisation and fall in input resistance. This effect, a consequence of increased non-junctional membrane permeability to Na^+ (refs 18 and 19), was particularly pronounced when the aequorin glow was diffuse; the depolarisation then paralleled closely the onset of uncoupling. Thus, the question presented itself of whether $[Ca^{2+}]_i$ or depolarisation is the primary cause of uncoupling. To resolve this question, we injected Ca^{2+} while clamping the membrane potential at resting level with a feedback system. Uncoupling ensued none the less whenever the glow reached a junction. Depolarisation is thus clearly not necessary for uncoupling.

In a complementing series of experiments, the cells were exposed to high K (90 mM) medium. Within 2–5 min of application of K medium, the membrane potentials fell to near zero or overshoot zero by 5–10 mV. The depolarised cells nevertheless stayed well coupled much longer, in some cases for several hours. Eventually, and rather abruptly, the cells uncoupled; and this was associated with an abrupt rise

in $[Ca^{2+}]_i$. Depolarisation is thus also not sufficient for uncoupling.

In conclusion, the cytoplasmic free Ca concentration in the domain of the junction seems to determine the permeability of the junction. We do not know the mechanism by which the calcium ion alters the permeability. Conceivably Ca^{2+} binds to the junctional membrane changing the conformation of the junctional cell-to-cell diffusion channels². We favour this simple notion, because as our results show, the permeability change is readily reversed when the normal $[Ca^{2+}]_i$ is restored in cells with normal Ca-pumping and Ca-buffering ability.

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BIRGIT ROSE

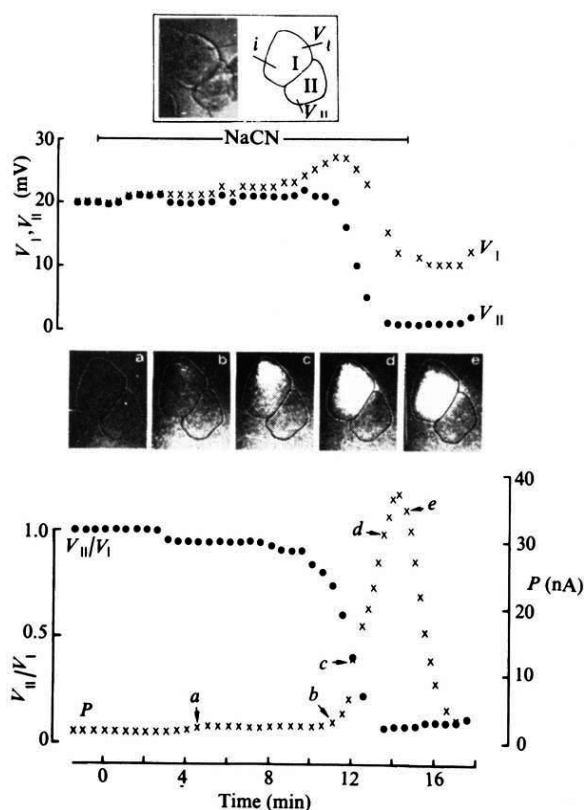
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Fig. 4 Cyanide. Cells in Ca-free medium. Exposure to 5×10^{-3} M sodium cyanide (top signal). Time correspondence of television pictures *a–e* is marked on the *P* curve. $i = 4 \times 10^{-8}$ A. Cell I depolarises as *P* rises, reaching zero membrane potential at time 17 min.



Modification of a model membrane structure by embedded photochrome

PHOTOREGULATED processes are of interest in diverse biochemical phenomena such as photosynthesis, vision, phototropism and phototaxis. The capture of a photon of appropriate energy, as the first event, triggers, or is followed by, subsequent steps such as electrical potentials or enzyme activity. In this respect, Erlanger and co-workers¹ have shown that some processes such as the catalytic activity of certain enzymes, which are intrinsically insensitive to light, may be modulated or regulated by the presence of appropriate photochromic molecules.

The primary process of photon capture in vision, photosynthesis and perhaps other related phenomena, is the absorption of light energy by 'sensor' molecules. Frequently, such 'sensor' molecules form part of, and are embedded in, membranes. The delineation of the steps starting from photon absorption by the membrane-embedded molecule and ultimately leading to the final response is of considerable importance. Such photo-induced processes may occur by means of a direct link between the 'sensor' molecule and the proteins (or other molecules) connected with the response, with the membrane providing an essentially inert milieu; or they may involve mediation by the membrane in which the 'sensor' molecule is incorporated.

An investigation into the plausibility of the latter proposition would involve monitoring the changes that might occur in the membrane structure on photon absorption by the 'sensor' molecule; in addition, an assay of the activity

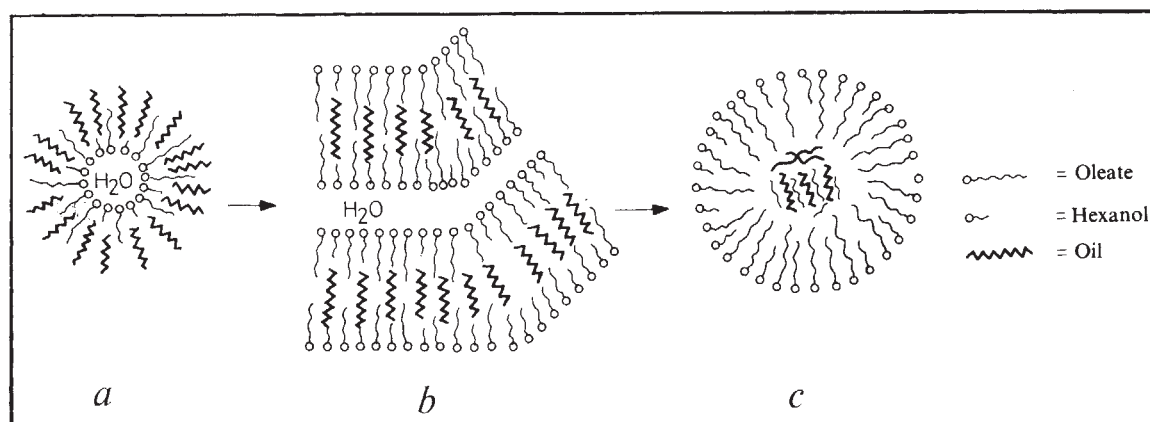


Fig. 1 Molecular organisation of the water-oil microemulsions. *a*, *c*, clear dispersions of water in oil (0–0.6), and oil in water (water-oil ≥ 1.2), respectively. *b*, Birefringent lamellar multi-bilayer system (water-oil = 0.6–1.2).

of a membrane-bound protein (or enzyme) would be desirable. Photo-induced changes in the conformation of a model membrane have been observed by Verma *et al.*², who studied the electron spin resonance of a spin-labelled phospholipid multibilayer film in the presence of incorporated retinal. We report here preliminary results on photo-induced conformational changes in a model membrane, and concomitant changes that occur in the esteratic activity of α chymotrypsin, incorporated in the model membrane.

Our model membrane system is the microemulsion obtained by the dispersion of water and hexadecane using the amphipathic potassium oleate as the emulsifier and hexanol as the cosurfactant. The properties of such systems have been shown by Shah³ to be relevant to membranes. Water-hexadecane (0.7:1.0 to 1.3:1.0 (v/v)) results in a birefringent phase in which the molecular organisation is as shown in Fig. 1*b*. Compositions with lower ratios yield clear dispersions of water in oil (Fig. 1*a*), whereas higher ratios (1.2:1.0 to 1.4:1.0) result in clear dispersions of oil in water (Fig. 1*c*). The relevant region is the intermediate water-oil composition depicted in Fig. 1*b*, providing a model for membranes as has been verified by several physical measurements⁴. We chose this region because it is structurally similar to liposomes, easily monitored by electrical resistance measurements, and, being in the liquid phase, quantitative spectral measurements and enzyme assays can be conveniently done. The response of this oil-water lamellar system to drugs and to electrolytes is similar to those of liposomes and of other membrane models³.

The photosensor molecules chosen were azobenzene and azobenzene-*p*-carboxylic acid methyl ester. Both these compounds are known⁵ to undergo reversible isomerisation from *trans* to *cis* conformations and back again on illumination with photons of appropriate wavelengths. The photoisomerisation produces long lived isomer species which relax with time into the equilibrium isomer population of the predominantly *trans* form. In a typical experiment, a small amount of the photochrome was added to the model membrane (photochrome-hexadecane molar ratio 1:300) and the electrical resistance of the membrane followed using the method of Shah⁴. Measurements were made on the system over a wide range of water-oil compositions, and in the presence of the photochrome in its equilibrium isomer population, in its *cis* form and the *trans* form, each of which is produced by flooding the membrane containing the photochrome with light of appropriate wavelength for several minutes. The variation in the resistance of the microemulsion as a function of the water-oil ratio is shown in Fig. 2. We were particularly interested in the region where the system adopts a liquid crystalline lamellar molecular arrangement. The

arrow A in Fig. 2 shows the resistance changes that occur in the model membrane when the photochrome is in its equilibrium isomer population (on the curve), on converting it completely to the *trans* isomer (illumination with a 250 W mercury lamp using a Corning CS 373 filter 400–500 nm window for 8 min, resistance at lower end of arrow A), and photoisomerising it completely into the *cis* form (using same lamp with a Corning CS 760 filter, 300–400 nm window for 8 min, resistance corresponds here to the top of arrow A). On standing, the photochrome reverts to its equilibrium isomer ratio and the resistance of the system also returns to the original value on the curve. Such changes in the resistance are reversible over several cycles of photoisomerisation, and occur both with azobenzene or the azobenzene ester as the photochrome. The photochrome molecule, itself non-conducting, does not contribute to the resistance, that is, the variation in resistance with composition shown in Fig. 2 is essentially the same with or without the photochrome, in the absence of light. Also, there is no change in the resistance of the system on illumination in the absence of the photochrome, thus discounting the possibility of photoinduced perturbation of the microemulsion structure by itself. Changes in the resistance of the type shown in arrows A and B of Fig. 2 also occur, but less significantly, in the clear regions of the microemulsion.

The changes in the resistance may result from a perturbation in the molecular organisation within the lamellar model membrane induced by the conformational change occurring in the incorporated photochrome on photoisomerisation. This interpretation is supported by the reversible changes from the birefringent phase to the clear phase and back, observed on the *trans* to *cis* to *trans* interconversion of the photochrome in the microemulsion of water-oil composition (0.7:1.0) corresponding to the borderline situation between clear dispersion and the lamellar phase. Shah⁴ has observed anomalous changes in the resistance of such lamellar water-hexadecane system on the introduction of anaesthetic drugs, and has interpreted them as resulting from perturbations in the molecular organisation within the lamellae. Our results and interpretation agree with those of Verma *et al.*² who monitored the electron spin resonance signals of a spin label incorporated in a similar bilayer model system. It therefore seems possible to induce perturbations in the structure of a model membrane by altering the geometric shape or conformation of an imbedded photochrome molecule.

To investigate whether such membrane structural perturbations could result in a change in the activity of an enzyme incorporated in the membrane, we monitored the ester hydrolytic activity of α chymotrypsin dissolved in the

membrane containing azobenzene and the azobenzene ester separately. We chose the lamellar region of the emulsion, and the simplified Bender-Kezdy method of assay⁶ with *p*-nitrophenyl acetate as the substrate. The activity of the enzyme was monitored by incubating the solution of the enzyme in the membrane with the substrate in the presence of azobenzene for 10–15 min and spectrophotometrically following the liberated *p*-nitrophenol absorbance with time (see Fig. 2). The photochrome–azobenzene carboxylic ester was unsuitable since it was found to directly bind to chymotrypsin. Azobenzene showed no appreciable binding or modification of the catalytic activity of chymotrypsin in an aqueous buffer solution control. If the activity of chymotrypsin towards *p*-nitrophenyl acetate in the model membrane in the presence of the equilibrium population of azobenzene is taken as unity, the activity when the azobenzene was exclusively in the *cis* form (photoisomerised at 300–400 nm for 10 min) was found to be fourfold greater, whereas the activity of the enzyme in the presence of exclusively *trans* photochrome (irradiation at 400–500 nm for 8 min) was about 0.6. Since azobenzene does not seem to bind directly to the protein in our system or modify its catalytic activity in the control, and since chymotrypsin itself is insensitive to light⁷, these results have been interpreted as follows. Photoisomerisation of the 'sensor' photochrome causes a rearrangement of the membrane structure, and this structural change results in a slight conformational perturbation in, and therefore the activity of, the membrane-bound protein.

These model studies may be of relevance to the photo-physical changes that occur in the rhodopsin molecule, on bleaching, in the retinal rod outer segment disk membranes. On bleaching, the rhodopsin molecule 'sinks' into the membrane⁷. It is possible that photoisomerisation of the retinal molecule alters the molecular organisation of the

disk membrane in such a way as to accommodate the protein deeper into the membrane.

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Effect of temperature on immune damage of liposomes prepared in the presence and absence of cholesterol

SINCE the report by Kinsky¹ that liposomes containing lipid haptens could be damaged by activated complement, it has become generally accepted that the lytic attack mechanism of the complement system acts on the lipid matrix of the cell membrane. In spite of the accumulation of experimental observations² concerned with liposomal damage by activated complement, there is little detailed information about the mechanism by which the damage occurs. Inoue and Kinsky³ suggested that complement did not damage liposomes as a result of degradation by 'phospholipase' (see also ref. 4). It was therefore postulated that the lytic action of complement may resemble that of 'detergent'^{2–4}. Hydrophobic regions in the terminal complement components may penetrate into lipid bilayers, diminishing the non-polar intermolecular attractive forces between lipids. Müller-Eberhard *et al.*⁵ proposed a model for the molecular assembly of the C5–9 complex on the surface of cells under attack by complement. They suggested that complete assembly of the C5–9 complex augmented the detergent effect of C5.

We reported previously that liposomal damage induced by lysolecithin⁶, polymyxin B (ref. 7) and Prymnesin⁸ required certain states of fluidity in lipid bilayers, but it is not known whether complement-induced liposomal damage is also influenced by fluidity of bilayers. We report here that, using dipalmitoyllecithin liposomes, the complement action requires some fluid conditions of membranes.

Immune-sensitive liposomes were prepared using the methods of Kinsky *et al.*⁹ and dipalmitoyllecithin liposomes without cholesterol were prepared as described previously¹⁰. Forssman antigen isolated from horse spleen was donated by Dr A. Makita, Hokkaido University. Antisera against Forssman antigen were prepared by intravenously injecting sheep erythrocyte membranes into rabbits. The release of glucose marker was enzymatically assayed as described previously.

Dipalmitoyllecithin liposomes (no cholesterol) sensitised with Forssman antigen were incubated with excess amounts of antiserum and guinea pig serum (complement) at various temperatures. Damage of the liposomes by antibody and complement was markedly dependent on temperature (Fig. 1a). Below 15 °C, the reaction was not detectable, but glucose release was observed at higher temperatures. Up to 35 °C, the reaction increased with temperature. Since spontaneous release of glucose occurred from the liposomes above 37 °C (ref. 10), which resulted from the so-called phase separation of lipid bilayers, the exact temperature dependence of complement-induced permeability change could not be examined above this temperature. The

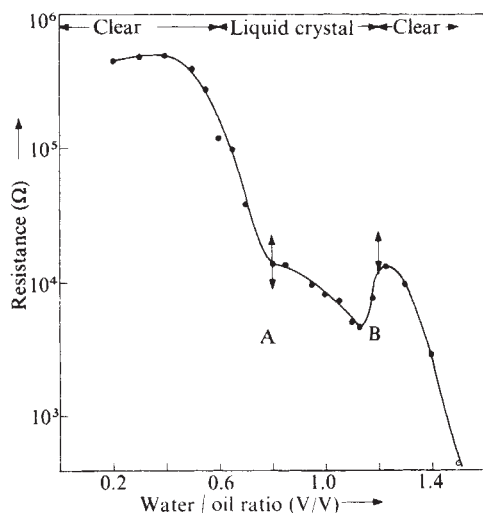


Fig. 2 Variation of the electrical resistance of the water–oil dispersions with composition. Photochrome (1.5 mg) is added to a mixture containing 1 ml hexadecane, 0.2 g potassium oleate and 0.4 ml hexanol. The amount of water is varied to obtain appropriate compositions. The resistance was measured using a Philips PR 9500 conductivity bridge at 1 KHz, with platinum electrodes 0.8 cm apart. Temperature 28 ± 1 °C. For the assay, 0.6 mg of *p*-nitrophenyl acetate in 0.2 ml hexanol was added to a mixture of 0.38 ml hexanol, 1.0 ml hexadecane, 1.0 ml H₂O, 3.2 mg azobenzene, 0.2 g potassium oleate and 3 mg chymotrypsin (σ) that has been irradiated for 10 min at the chosen λ . The A_{400} of the released *p*-nitrophenol was measured at 90 s intervals and the rate of release was calculated from the linear (later) portion of the release curve. The reference cuvette contained the same solution (minus substrate) and was taken through all the steps as the sample. Path cells (1 cm) were used in a Beckmann DU instrument (28 ± 1 °C).

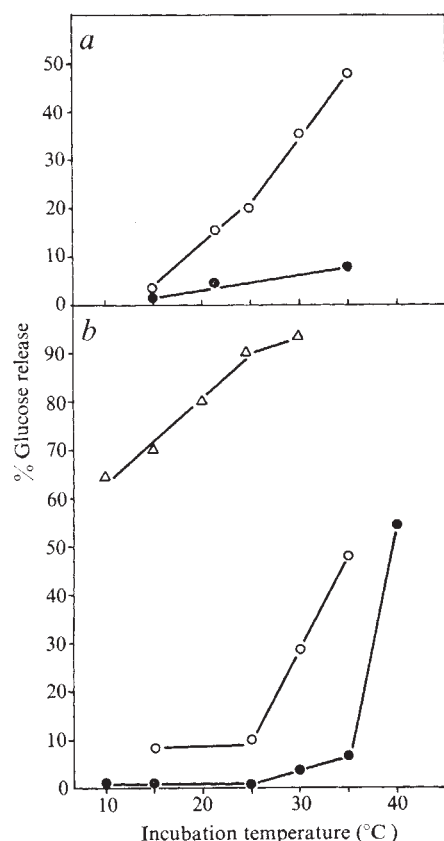


Fig. 1 Temperature dependence of liposomal damage by activated complement, methylated bovine serum albumin and Triton X-100. Liposomes were prepared from mixtures of dipalmitoyllecithin and dicetyl phosphate (1:0.1) with or without 10 μg Forssman antigen per μmol phospholipid. *a*, Liposomes without antigen (●) and with antigen (○) were incubated with 11 μl anti-Forssman antiserum and 34 μl guinea-pig serum at various temperatures for 30 min. *b*, Liposomes without antigen were incubated alone (●), with 35 μg Triton X-100 (Δ) or 12 μg methylated bovine serum albumin (○) for 10 min at various temperatures.

liposomes without Forssman antigen released little glucose throughout the temperature range 10–35 °C.

The effect of antiserum concentration on the loss of marker from dipalmitoyllecithin liposomes which were prepared with a certain amount of Forssman antigen (10 μg per μmol phospholipid) is shown in Fig. 2. The experiments were performed at 35 °C. From these curves, we determined the amount of antiserum required for glucose release under conditions in which antigen content and concentration of complement source are not rate limiting. Liposomes prepared with dipalmitoyllecithin and dicetyl phosphate showed almost the same sensitivity towards antiserum as those made with a mixture of dipalmitoyllecithin, dicetyl phosphate and cholesterol (Fig. 2). The amount of fresh guinea pig serum (complement) necessary for marker release was determined

by the same procedure. The results indicated that sensitivities of liposomes both with and without cholesterol against complement were not significantly different (Fig. 3). We therefore concluded that cholesterol does not have any influence on the sensitivity of liposome to antiserum and complement.

There was no difference in sensitivity towards antiserum and complement between liposomes of dipalmitoyllecithin, dimyristoyllecithin and egg lecithin, whereas rather different sensitivity was reported between egg lecithin and beef sphingomyelin liposomes¹¹. Complement-dependent glucose release from dipalmitoyllecithin liposomes, egg lecithin liposomes and dimyristoyllecithin liposomes, all of which contained 50 mol % of cholesterol, was not as influenced by incubation temperature as the release from dipalmitoyllecithin liposomes without cholesterol (Table 1).

Incorporation of cholesterol into dipalmitoyllecithin liposomes has been shown to have complicated effects on the permeability of glucose; it may give a dual effect, that is, the condensing and fluidising effect on bilayers of dipalmitoyllecithin. Dipalmitoyllecithin liposomes without cholesterol showed very little reactivity with antiserum and complement at 21–23 °C, whereas those with cholesterol were damaged in the same reaction conditions (Table 1). The effects of cholesterol incorporation on immune damage of liposomes may be caused by the fluidising effect. Introduction of cholesterol generally suppressed the sensitivities of liposomes towards various lytic reagents such as lyso-

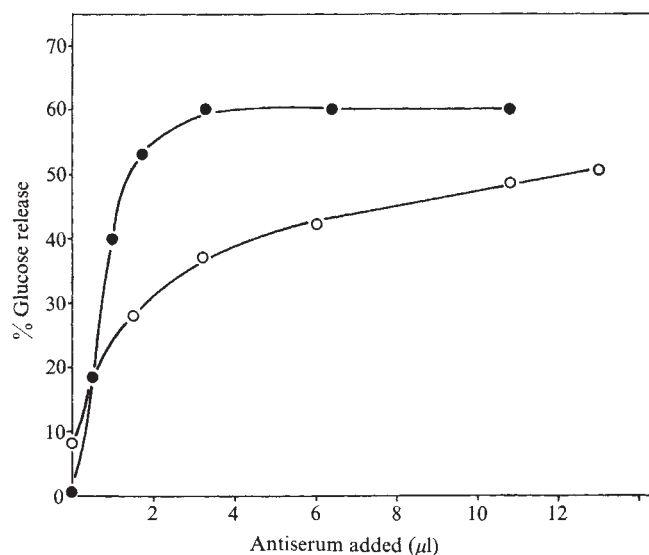


Fig. 2 Effects of antiserum concentration on dipalmitoyllecithin liposomes prepared with Forssman antigen. Liposomes containing 10 μg per μmol phospholipid were prepared with and without cholesterol as described in the legend to Table 1. Compositions of liposomes were as follows: dipalmitoyllecithin (DPL), dicetyl phosphate (DCP) and cholesterol (Chol) in the molar ratio of 1:0.1:1 (●); dipalmitoyllecithin (DPL) and dicetyl phosphate (DCP), (○) 1:0.1:1.

Table 1 The specific damage of liposomes containing antigen by antibody and complement

Lecithin	C14:0		C16:0		Egg	
Cholesterol-lecithin (molar ratio)	1.0	1.0	1.0	0	1.0	1.0
Incubation temperature (°C)	21–23	35	21–23	35	21–23	35
Serum added	% Trapped glucose released within 30 min					
None	0.25	1.52	0	1.87	1.89	8.03
GPS + AS	60.7	68.5	53.3	58.9	16.5	47.2
Heated GPS + AS	2.29	1.52	1.40	0.46	1.42	7.56
GPS	0	0.38	0	0	0.95	8.50
					3.85	3.36

Liposomes were prepared from various lecithins and 10 μg Forssman antigen per μmol phospholipid without or with cholesterol. Glucose release was determined after 30 min incubation at 21–23 or 35 °C with various sera as indicated. The amounts of each serum added were as follows: guinea pig serum (GPS), 35 μl; heated guinea pig serum (heated GPS), 35 μl; rabbit anti-Forssman serum (rabbit AS), 30 μl.

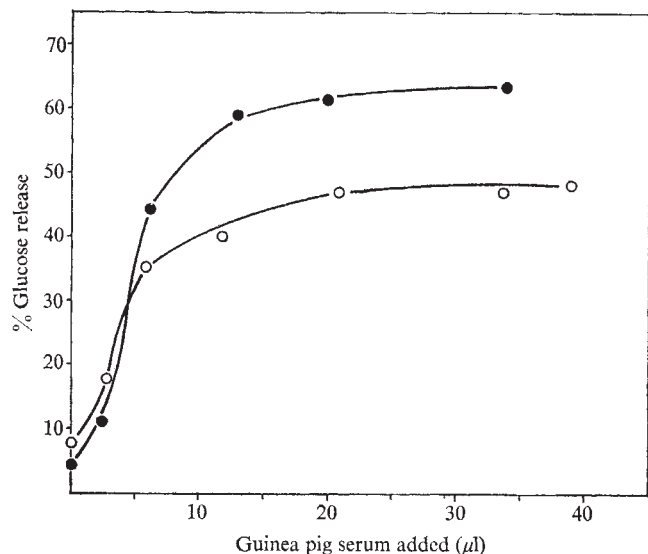


Fig. 3 Effects of guinea pig serum concentration on dipalmitoyllecithin liposomes prepared with Forssman antigen. Liposomes containing 10 μg per μmol phospholipid were prepared with (●) and without (○) cholesterol. The liposomal compositions were as described in Fig. 2. Glucose release was determined after liposomes were incubated with varying amounts of guinea pig serum in the presence of 11 μl antiserum at 35 °C for 30 min.

lecithin⁶, polymyxin B (ref. 7) and some basic proteins¹². The condensing effect of cholesterol may interfere with the penetration of these molecules into bilayers, but the suppressive effect of cholesterol could not be observed in the case of complement-induced damage of liposomes. Dipalmitoyllecithin liposomes with 50 mol% of cholesterol are among the most resistant liposomes yet reported, that is, they resist the action of Triton X-100 (ref. 10), lysolecithin⁶, polymyxin B (ref. 7) and methylated bovine serum albumin (T.K. and K.I., unpublished). Activated 'hydrophobic' complement components may penetrate into such 'highly condensed' membranes.

Methylated serum albumin is one of the basic proteins with ability to damage liposomes. The mechanism by which the protein damages lipid bilayers may be considered as follows¹³: interaction of positively-charged residues of proteins with negatively-charged components of the membranes; change in conformation of the proteins; penetration of exposed 'hydrophobic' regions of proteins into the bilayers. The reaction of dipalmitoyllecithin liposomes with methylated bovine albumin also depends markedly on the incubation temperature (Fig. 1b). Temperature dependence of methylated albumin-induced liposomal damage was rather similar to that of immune damage of the liposomes. On the other hand, the detergent Triton X-100 could affect the liposomes to almost the same degree over the temperature range 10–35 °C. Molecules of Triton X-100 can easily penetrate into dipalmitoyllecithin bilayers, whereas protein molecules can not penetrate into bilayers of membranes whose arrangement is too condensed. With increased temperature, arrangement of hydrophobic regions of the membrane becomes loose and allows proteins to penetrate. It is quite conceivable to assume that the mechanism of membrane damage by activated complement is rather similar to the mechanism of methylated albumin-induced membrane damage. The lower transition or pretransition (about 35 °C) was reported¹⁴ on dipalmitoyllecithin. The pretransition has been associated¹⁴ with an increase in mobility of the polar head portion of the lipid. The increase in mobility of the polar head portion may allow proteins to penetrate into bilayers.

The effect of proteins in altering the phase transition characteristics must also be considered. Some proteins could

act as modifiers of the lipid phase transitions. Addition of cytochrome C has been reported to lower the transition temperature of lipids¹⁵. Our experimental systems, antibody and activated complement components, may also modify the lipid phase transitions. It is possible that the effect of activated complement in lowering the transition temperature of dipalmitoyllecithin may be responsible for our results. If true, it should be noted that the binding of antibody molecules could not modify the lipid phase transition.

Our results suggest that liposome damage induced by complement requires some fluid condition of membrane. Cholesterol does not play an important role in the binding of antibody to antigen on the membrane not in the membrane damage by complement components. Simple model systems of immune lysis could be established to investigate the mechanism.

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Appearance of β globin synthesis in erythroid cells of Ferrara β⁰-thalassaemic patients following blood transfusion

HOMOZYGOUS β-thalassaemic subjects from the Ferrara region characteristically do not synthesise β globin^{1,2}. Some of us have presented previously data which suggest that the addition of ribosome-free supernatant isolated from red blood cells of normal adult individuals or from subjects with homozygous haemoglobin S to Ferrara thalassaemic ribosomal material induces β^A globin synthesis³; these results suggested that this *de novo* synthesis was not associated with free normal β^A globin mRNA. From these findings it has been concluded that the mutation of the Ferrara β thalassaemia does not reside in the gene for β globin, which is in fact transcribed into normal β globin mRNA; but in a gene coding for a previously unrecognised factor, indispensable for β globin translation. Here we report data which suggest that the reticulocytes from these patients synthesise β globin following normal blood transfusion.

Table 1 shows the amount of radioactivity incorporated into α, β and γ globin in the reticulocytes of a homozygous β thalassaemic subject from Ferrara, before and 5, 17, and 24 d after receiving the first blood transfusion. α and γ globin synthesis varied only slightly, while the incorporation of radioactive leucine into β globin, undetectable before the treatment, became clearly demonstrable after blood transfusion.

As a result of the post-transfusional appearance of β globin synthesis, the ratio of α- to non-α-globin synthesis decreased from 2.6 to more normal values. The specific activity of purified

Table 1 ^3H -leucine incorporation into α , β and γ globin (separated as in refs 7 and 8) by 0.5 ml of packed red blood cells of a homozygous β -thalassaemic subject from Ferrara, before and after blood transfusion

	α globin	Total radioactivity (c.p.m.) β globin	γ globin	Total globin synthesis (c.p.m.)	% of β globin synthesis	^3H -non- α -globin
Before transfusion						
30 d before	14,763	0	5,529	20,292	0	2.6
1 d before	15,711	0	6,015	21,762	0	2.6
After transfusion						
5 d after	13,905	4,095	5,520	23,520	42.5	1.4
17 d after	13,440	7,091	5,940	26,571	54.7	1.0
24 d after	15,051	5,514	5,793	26,358	48.7	1.3

0.5 ml of washed red blood cells from a homozygous β -thalassaemic subject from Ferrara, obtained before and after the first blood transfusion at the days indicated, were incubated with ^3H -leucine for 2 h at 37°C , as previously reported¹. After incubation the cells were lysed and the stromal material removed by centrifugation. Globins prepared from stroma-free haemolysates by acid acetone precipitation⁴ were separated into their individual chains by CM cellulose column chromatography^{7,8}. The chromatographic pattern was continuously monitored with a Gilford recording Spectrophotometer (Model 2400) at 280 nm and fractions of 2.5 ml were collected. Radioactivity determinations were on 0.2 ml from each fraction, in Bray's solution¹³, with a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3003), efficiency for tritium 49%.

Total incorporation into α , β and γ globin was calculated on the radioactivity pattern, drawn after background subtraction.

^3H -non- α -globin synthesis = ^3H - β -globin + ^3H - γ -globin.

β globin, obtained from isolated haemoglobin A, was maximal approximately 13 d after transfusion (see Table 2).

The characterisation of the radioactive protein appearing in the β globin region was carried out in a β^0 -thalassaemic subject studied 20 d after he had received the first blood transfusion. Following a standard red blood cell incubation with ^3H -leucine, haemoglobin A was isolated by chromatography on Amberlite CG-50⁴ and converted into globins⁵. Globins were purified further by chromatography on Sephadex G-200⁶. Finally the β globin chains were isolated^{7,8}.

The isolated β globin chains were digested with trypsin and the resulting peptides separated by column chromatography⁹. The baseline radioactivity was measured by determining the number of counts in four regions of the chromatogram, one before βT_3 (B1), one between ammonia and βT_8 (B2), one before βT_6 (B3) and one after βT_{15} (B4). The measured radioactivity (c.p.m.) in the fractions was: B1 = 39; B2 = 99; B3 = 77 and B4 = 50. The fractions of the leucine-containing peptides βT_1 , 2, 3, 5, 9 and 14, and of peptide βT_{13} , were separately pooled, lyophilised and purified further (see legend to Table 3).

After purification of the leucine-containing peptides βT_1 , 2, 3, 5, 9, and 14 their specific activities were determined (Table 3). Four non-leucine-containing peptides were also studied; three of them (βT_6 , 7 and 15) were analysed directly after the first chromatography, while peptide βT_{13} was purified further. The purification of peptides βT_1 , 2, 5, 9 and 14 was successful, with a good yield in terms of absorbance and radioactivity; peptides βT_3 and 13, although pure by amino acid analysis, were recovered in amounts too low to give acceptable information.

The analysis of the results (Table 3) was limited to peptides βT_1 , 2, 5, 6, 7, 9, 14 and 15, which nevertheless represent a sizeable amount of the β globin molecule and are good samples of the entire chain, being located at the beginning, in the middle and at the end of the molecule. As the radioactivity is present in the five leucine-containing peptides in amounts proportional to the amino acid content, and practically absent in the peptides without leucine, considering the amount of peptide recovered (see Table 3), it can be concluded that the radioactive material analysed in the β^0 -thalassaemic subject under investigation after blood transfusion was β globin. Furthermore, the calculated specific activity of leucine in the original isolated globin was $518.8 \text{ c.p.m. } \mu\text{mol}^{-1}$ (assuming 1.0 absorbance unit = 1 mg of protein), close to that of the leucine in the isolated peptides.

Figure 1 shows the percentage of β globin synthesis, relative to total non- α -synthesis, demonstrated in 65 subjects examined at different times after blood transfusion. β Globin synthesis was present in all subjects except two; in these patients β -synthesis was not present after the first blood transfusion, but appeared after the second. As shown in Fig. 1, the maximum

β globin synthesis occurred approximately between the 15th and the 20th day after blood transfusion. Over the same period and in the same subjects the ratio of α -globin synthesis to non- α -globin synthesis decreased from the usual pretransfusion values to unity (see Fig. 2). In two patients, globin synthesis by intact erythroid cells was determined following injection of plasma from two normal adult individuals; no β globin synthesis was detected in either case.

The kinetics of the appearance of β globin synthesis after blood transfusion derived from Table 1 and Fig. 1 indicates that haemoglobin A synthesis occurs in the patient's cells and not in the transfused red cells. In fact in the latter case β globin synthesis should be maximal immediately after transfusion and rapidly disappear during the days following.

The occurrence of β globin synthesis in thalassaemic reticulocytes has been confirmed in four experiments in which, 20 d after blood transfusion, the erythrocytes of the donor (of O group) were separated from the erythrocytes of the patient (of A group) by means of lectin precipitation^{11,12}. Practically all β globin synthesising capacity was found in association with the non-agglutinated thalassaemic red blood cells. In the same four samples the amount of non-radioactive β globin, relative to

Fig. 1 Relative synthesis of β globin as determined by CM cellulose column chromatography in 55 homozygous β^0 -thalassaemic subjects from Ferrara, following blood transfusion. No β globin synthesis was detected either before or 2–3 months after transfusion. Methods as Table 1.

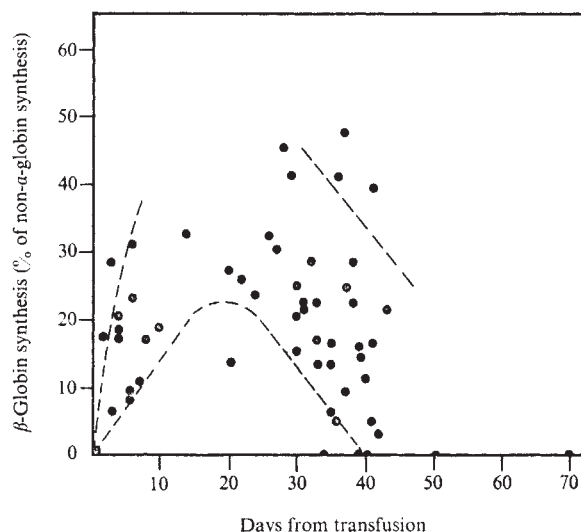


Table 2 Incorporation of radioactivity into purified β -globin by the reticulocytes of a homozygous β -thalassaemic patient from Ferrara, before and after blood transfusion

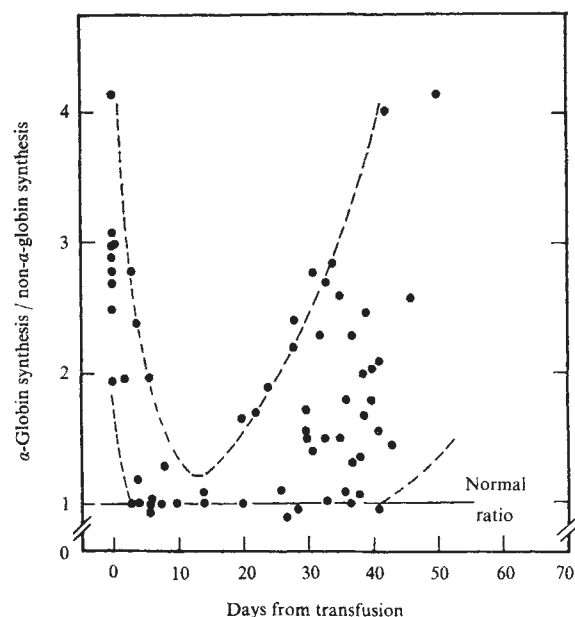
Days from transfusion	Specific activity (c.p.m. $\times 10^{-3} \times A_{289}^{-1}$)
Days before	
5	0
Days after	
5	1.18
10	1.69
17	1.77
24	1.38

1.0 ml of washed packed red blood cells, obtained at the days indicated, were incubated with ^3H -leucine for 2 h, at 37 °C. Stroma-free lysates, obtained as reported in Table 1, were submitted to Amberlite CG-50 chromatography⁴ and the purified haemoglobin A submitted to CM cellulose column chromatography⁷⁻⁸ for chain separation. The subject is the same as that referred to in Table 1.

total non- α -globin, measured as absorbance at 280 nm after CM cellulose column fractionation, ranged from 12% to 21%.

Control experiments showed that the above technique effectively discriminated between red blood cells of different groups. The lectin agglutination experiments, as well as showing that β globin is synthesised by thalassaemic erythroid cells, exclude the possibility that the appearance of β globin synthesis may be a result of bone marrow graft and proliferation of immature red blood cells, possibly present in the peripheral blood of the donor.

The data reported here strongly suggest the appearance of β globin synthesis in Ferrara thalassaemic erythroid cells after blood transfusion; it is possible that some thalassaemic subjects previously reported in the literature as " β^+ " may have been transfused β^0 patients. Our findings suggest that the specific inducer of β globin synthesis, previously shown to be present in normal red blood cells³, can cross the thalassaemic red cell membrane and replace the absent or non-functioning thalassaemic factor in inducing β globin mRNA translation. In line with this suggestion is the fact that the maximum of β globin synthesis corresponds approximately with the maximum of red cell destruction. Red cell lysis occurs in the reticuloendothelial

**Fig. 2** Synthesis of α -globin relative to non- α -globin in 55 homozygous β^0 -thalassaemic subjects from Ferrara, following blood transfusion.

system, which is well represented in the spleen, but also in the bone marrow; in the latter therefore the maturing thalassaemic red blood cells could be exposed to a relatively high concentration of normal red cell cytoplasm and, consequently, of the postulated normal β globin 'inducer'.

We cannot distinguish at the moment whether the *in vivo* induced β globin synthesis is directed by transfused or by thalassaemic β globin mRNA. So far we have been unable to obtain and transfuse to Ferrara thalassaemic patients blood from subjects homozygous for a mutant stable β globin (as in haemoglobin E or haemoglobin C), which would discriminate between these two possibilities. Nevertheless the short survival time of the protein synthesising capacity in reticulocytes, together with the presence in Ferrara thalassaemic red blood

Table 3 Incorporation of radioactivity into the tryptic peptides of purified β globin isolated from a homozygous β^0 -thalassaemic subject 20 d after blood transfusion

Leucine-containing peptides	Molecules of leucine per peptide	Recovery of leucine (μmol)	Recovery of radioactivity (c.p.m.)	Specific activity (c.p.m. per μmol leucine)
βT_1	1	1.305	760	582
βT_2	1	1.271	771	606
βT_3	1	0.059	36	607
βT_5	1	0.325	150	461
βT_9	4	2.588	1,615	624
βT_{14}	1	1.220	823	674
Non-leucine-containing peptides		Recovery of peptide (μmol)		
βT_6	0	2.032	151	—
βT_7	0	2.000	39	—
βT_{13}	0	0.075	43	—
βT_{15}	0	2.109	90	—

The fractions containing the leucine-containing peptides βT_1 , 2, 3, 5, 9 and 14, and of peptide βT_{13} , were separately pooled, lyophilised and purified further as follows: βT_1 , 2 and 14 were purified by column chromatography on Aminex 50W-X2, pyridine-acetate linear gradient from pH 3.1, 0.2 M to pH 5.0, 2.0 M; βT_3 and 13 by two successive treatments on Aminex 50W-X2, pyridine-acetate linear gradient from pH 3.1, 0.2 M to pH 5.0, 2.0 M, and a third column chromatography on Aminex AG1-X2, using the elution system described by Schroeder and Robberson¹⁰; βT_5 by column chromatography on Aminex 50W-X2, pyridine-acetate linear gradient from pH 2.75, 0.1 M to pH 4.25, 1.25 M. βT_9 by column chromatography on Aminex 50W-X2, pyridine-acetate linear gradient from pH 3.1, 0.2 M to pH 4.25, 1.25 M. The profile of the various peptides after rechromatography was continuously monitored at 570 nm. The fractions corresponding to the various peptide peaks were pooled and lyophilised. Amino acid analysis was done on 1/40th of the lyophilised material to check the purity of the peptides after rechromatography, and to determine the leucine content necessary for the specific activity determinations. The residual material was used for radioactivity measurement. The amino acid analyses proved the purity of the various peptides; the radioactivity blanks of the rechromatographies ranged between 15 and 40 c.p.m.

cells of untranslated β globin message³ are good indications that the post-transfusional β globin synthesis is directed by thalassaemic β globin mRNA. In both cases, however, the inducer of β globin synthesis has to become available within the thalassaemic erythroid cells (in which this molecule is lacking or not functioning³) to permit β globin synthesis.

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Neurosecretory cells in insect brain and production of hypoglycaemic hormone

INSECTS have a hyperglycaemic hormone, produced by neurosecretory cells of the corpus cardiacum (CC). This hormone stimulates the production of the major blood carbohydrate trehalose from glycogen stored in fat body cells¹. No hypotrehalosaemic hormone has so far been reported and the level of blood trehalose has been thought to depend on: the secretory activity of the CC neurosecretory cells controlled by nerves from the brain^{2,3}; the relationship between production and consumption of trehalose, which in many insects, including flies, serves as fuel for flight. Also, *in vitro* experiments indicate that medium containing trehalose can inhibit the synthesis or liberation of trehalose from fat body cells, and it has been suggested that such feedback control is an important factor in the natural regulation of trehalose formation^{4,5}. It was therefore surprising to find that seven decapitated 6-d-old blowflies, *Calliphora erythrocephala*, not only remained alive for 30 h, during which locomotion and heart beat continued, but that their blood trehalose level had increased from 21.9 g l^{-1} ($\pm 4.09 \text{ s.e.m.}$) to 113.2 g l^{-1} ($\pm 9.07 \text{ s.e.m.}$).

There are several possible explanations for the extreme hyperglycaemia of the decapitated flies (Fig. 1). A hyperglycaemic factor could have been released from the CC, or from the corpus allatum (CA), or from the thoracic ganglion. Uncontrolled release might then be ascribed to removal of the brain. Alternatively, a hypoglycaemic hormone could be produced in the brain or secreted by some unknown endocrine organ controlled by the central nervous system.

Several surgical experiments (Table 1) were performed as previously described⁶, except that the use of Ringer was avoided. Close proximity of the functional elements (Fig. 1) makes it impossible to settle the matter by a single surgical experiment. Cardiectomy involves removal of the aorta wall adjacent to the CC, which contains axon terminals of the medial neurosecretory brain cells (MNC). Extirpation of the MNC, which seems to cause hyperglycaemia, inevitably injures adjacent tissue, and if nerves controlling the CC were affected, the effect might be caused by uncontrolled secretion of CC hormone. This possibility, however, is eliminated by the fact that hyperglycaemia also occurred after the CC had been removed. A similar effect results from severing of the cardiac-recurrent nerve (CRN). This operation inactivates the CC (refs 2 and 3); but it also prevents the release of brain hormone by interrupting the pathway to the neurohaemal organ (Fig. 1). Cutting of the ventral nervous connective is without significant effect, whereas allatectomy results in a slight hyperglycaemia within 20 h.

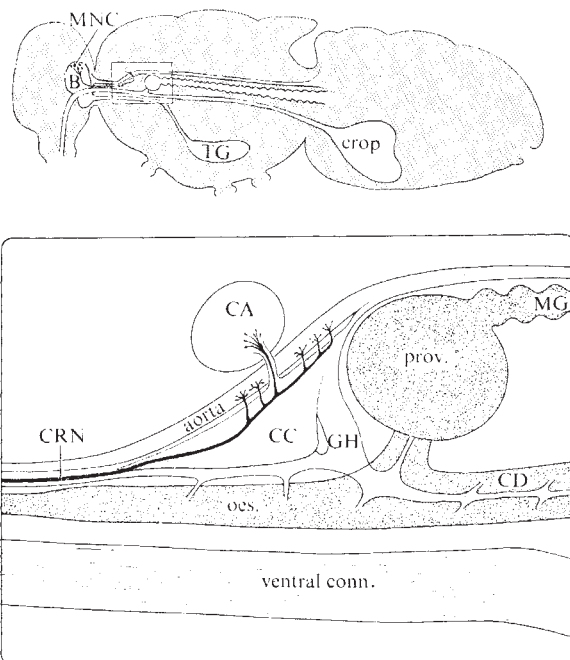


Fig. 1 Position of the main elements of the central nervous system and the neuroendocrine system in the blowfly, together with the anterior part of the aorta and of the alimentary canal with crop and crop duct. The retrocerebral endocrine complex (inset) illustrated as a simplified diagram below. B, Brain; TG, thoracic ganglion; CRN, cardiac-recurrent nerve; CA, corpus allatum; CC, corpus cardiacum; GH, hypocerebral ganglion; oes, oesophagus; MG, midgut; prov., proventriculus; CD, crop duct; MNC, medial neurosecretory brain cells. The main axis of the central nervous system, brain-ventral connective-thoracic ganglion, is severed by decapitation, as is the main axis of the neuroendocrine system, brain neurosecretory cells-CC-aorta wall-CA, since the CRN is severed. This compound nerve contains both neurosecretory and non-neurosecretory axons, the latter belonging to the stomatogastric nervous system. Axons from the MNC of the brain (aldehyde fuchsin positive) penetrate the CC (ref. 6) to terminate in the aorta wall dorsal to the CC. This region is their neurohaemal organ. Some MNC axons, however, enter the CA in adults⁷.

Table 1 indicates that the extreme hyperglycaemia is unlikely to be caused by any hyperglycaemic factor, from the ventral nervous axis or from the neuroendocrine axis. Rather, hyperglycaemia occurs after elimination of secretion, either of a brain hormone or CC hormone or both, and so seems to be caused by the lack of a depressing factor. The CC produces a hyperglycaemic hormone^{1-3,5,9}. The other possibility, that a brain hormone, transported to its neurohaemal organ near the CC by way of the CRN, has a hypoglycaemic function,

Table 1 Haemolymph trehalose level given as $\text{g l}^{-1} \pm \text{s.e.m.}$ before and after various surgical experiments on adult male and female blowflies

	Trehalose average (g l^{-1})	<i>n</i>	Significance <i>t</i> test
Female blood, before operation	27.5 ± 0.92	45	
Male blood, before operation	26.4 ± 1.22	60	
Pooled female and male before operation	26.8 ± 0.80	105	
Pooled female and male before operation*	26.1 ± 1.40	9	
MNC extirpated; 20 h after operation	63.6 ± 3.67	8	$P < 0.001$
Cardiacectomised; 20 h after operation	49.9 ± 4.07	9	$P < 0.001$
CRN severed; 20 h after operation	53.7 ± 3.24	29	$P < 0.001$
CRN severed; 24 h after operation*	56.9 ± 7.60	9	$P < 0.001^*$
Allatectomised; 20 h after operation	36.6 ± 1.96	42	$P < 0.001$
Ventral nerve cut; 20 h after operation	22.7 ± 2.83	8	$P \sim 0.1$

Samples from operated flies fed sugar and water only compared with pooled ($n = 105$) samples taken from females and males before operation (then 6 d old).

* Flies (3d old at operation) fed both meat, sugar and water before and during the 24 h before second blood sampling. They were treated separately for *t* test (difference between first and second blood samples).

seems likely, also in view of the ability of MNC extracts to revert the hyperglycaemia (Table 2).

The moderate hyperglycaemia produced by allatectomy may fit with observations indicating that the CA positively regulates the MNC (refs 10 and 11). When allatectomised adults were treated at the time of operation with *Cecropia* juvenile hormone (JH), applied topically on the abdominal cuticle, a trehalose concentration of only 7.5 g l^{-1} ($\pm 2.71 \text{ s.e.m.}$, $n = 9$) was found 20 h later. When JH had been applied 10 h after the operation, a trehalose level of 16.3 g l^{-1} ($\pm 1.58 \text{ s.e.m.}$, $n = 8$) was observed after another 10 h. Application of JH to ten non-allatectomised flies did not, however, affect blood trehalose concentration.

ever, normally derives from ingested sugar (sucrose) rather than from net fat body glycogen conversion to trehalose. Nine flies were given water but not sugar from emergence, and 12 h later the CRN was severed. At that time the blood contained 17.7 g l^{-1} trehalose ($\pm 0.72 \text{ s.e.m.}$). Blood samples taken 24 h after the operation, during which period they were still given water only, contained 13.0 g l^{-1} trehalose ($\pm 1.10 \text{ s.e.m.}$). They were then given sugar *ad libitum* and in 24 h the blood trehalose level then increased to 44.3 g l^{-1} ($\pm 2.70 \text{ s.e.m.}$).

In mosquitoes the MNC have been shown to regulate glycogen and fat synthesis^{14,15}. In *Calliphora* deprived of their MNC, the fat body cells contain much glycogen but only little fat⁸, and the same seems to hold for fat body cells after

Table 2 Effect of brain extracts (with and without MNC) on flies rendered hyperglycaemic by previous severing of the cardiac-recurrent nerve

	Trehalose average (g l^{-1})	<i>n</i>	Significance <i>t</i> test
CRN severed; 22 h after operation	52.3 ± 3.21	16	
2-3 h after injection of MNC extract	27.8 ± 9.66	8	$P < 0.02$
2-3 h after injection of brain extract	51.2 ± 7.02	7	

Each of 16 hyperglycaemic recipients was injected with a crude, freshly prepared extract (homogenate) in Ringer (made by means of a small Potter-Elvehjem type homogeniser) of either three groups of MNC from 7-d-old donors ($n = 8$) or of a similar amount of brain tissue from the same donors, not containing MNC ($n = 7$; 1 died).

In adults the neuroendocrine system is geared to make a relatively high concentration of blood trehalose available at the onset of flight. This may partly be caused by a low activity of the MNC. In larvae, in which high activity of the MNC and of the CA is linked with developmental events, the trehalose level would consequently be expected to be lower. Indeed, in Dipteran larvae including *Calliphora*¹³, trehalose has been reported to be very low or absent¹³. Preliminary analyses of trehalose concentrations showed $0.5\text{--}3.5 \text{ g l}^{-1}$ ($n = 6$) in second instar larvae, $2.8\text{--}10.2 \text{ g l}^{-1}$ in third instar larvae ($n = 9$), and up to 16 g l^{-1} in pupae ($n = 5$). Haemolymph trehalose was also determined in different developmental stages of the butterfly *Pieris brassicae* (Table 3). Again, the more active the MNC and the CA are known to be, the smaller is the content of trehalose in the blood.

Hyperglycaemia could be induced both in sugar-fed flies and meat-fed flies (Table 1). Increased blood trehalose, how-

severing the CRN. Thus, lipid catabolism may be accelerated as in diabetic vertebrates.

Hyperglycaemia was associated with blood hyperosmolarity ranging from 600–1200 mosmol (normal osmolarity 350–400 mosmol). This may be the cause of the polydipsia which developed on removal of the MNC or cutting the CRN: within 10–12 h the flies increased in weight from the normal 65–75 mg up to 130 mg. Extreme bloating resulted not only from distension of the crop, but also be increased haemolymph volume. Such polydipsia in blowflies has been described^{8,16}, but bursting at weak points of the cuticle often occurs during the night, and so may have passed unnoticed. In fact much blood is lost and by continued polydipsia haemolymph composition must be greatly disturbed. This, together with the hypertonicity, is bound to have harmful effects on most tissues. Since several abnormalities, which seem to result from lack of brain hormone, at least superficially resemble diabetic conditions in vertebrates, it was tempting to test whether insulin had any effect in blowflies (Table 4). No interpretation seems warranted until more knowledge of the endocrine system in blowflies has been obtained and it should be added that no significant effects of injection of insulin into normal intact flies has so far been found.

In conclusion, it is suggested that MNC have an important role in the regulation of carbohydrate metabolism. When they are removed or prevented from secretion, hyperglycaemia ensues, accompanied by blood hypertonicity, polydipsia, lipid catalysis and fatigue (decreased and slow locomotor activity, perhaps resulting from impeded uptake of trehalose by the muscles). These effects should be related to other findings on

Table 3 Haemolymph trehalose ($\text{g l}^{-1} \pm \text{s.e.m.}$) at different developmental stages of the butterfly *Pieris brassicae*

	Trehalose average (g l^{-1})	<i>n</i>
Third instar larvae	12.5 ± 1.39	7
Fifth instar larvae	16.9 ± 0.49	7
Non-diapausing pupae	14.5 ± 2.17	8
Diapausing pupae	32.4 ± 1.13	9
Adults	29.7 ± 4.17	9

Diapausing pupae (MNC resting) contain significantly ($P < 0.001$) more trehalose in the blood than non-diapausing pupae, in which the MNC are active.

Table 4 Effect of injecting 500–800 IU kg⁻¹ crystalline ox insulin (insulin) or zinc protamin insulin (ZPI) into hyperglycaemic flies (CRN previously severed)

	Trehalose average (g l ⁻¹)	n alive	n died
CRN severed 24 h earlier	53.5 ± 3.65	20	
18 h after injection of insulin	23.6 ± 5.28	7	1
18 h after injection of Ringer	57.1 ± 5.45	8	4
3 h after injection of insulin	41.5 ± 4.37	5	
15 h after injection of ZPI	37.8 ± 5.87	7	
3 h after second dose of ZPI	29.6 ± 4.28	7	

Calliphora-Ringer (1 µl) containing insulin, or Ringer alone (1 µl) was injected after the removal of superfluous blood. For about a minute after the insulin injection there were convulsions of the somatic musculature, and increased locomotor activity continued for up to 20 min. Otherwise, insulin and ZPI were well tolerated, and the mortality on the second day after severing the CRN was generally lower than in non-treated flies (of which 20–30% died on the second day). The trehalose level before injection was only determined for the first 20 flies (the first experiment and control), since the average value remained close to that in Table 1 (CRN severed).

MNC function and unless MNC hormone(s) has a diversity of functions, it should be determined which effects are secondary to which, when results of MNC extirpation are considered.

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Effect of dimethylnitrosamine on persistence of methylated guanines in rat liver and kidney DNA

DIMETHYLNITROSAMINE is a potent carcinogen in many species, inducing tumours of the liver, kidney and lung^{1,2}. It is now well established that the carcinogenic activity of this compound is caused by its metabolic transformation within susceptible tissues to a chemically reactive agent which methylates a variety of cellular macromolecules^{1–3}. In spite of the fact that metabolism of dimethylnitrosamine is eight times greater in the liver than in the kidney, thus the alkylation of macromolecules is eight times greater in liver than kidney, a single large dose of dimethylnitrosamine given to the adult rat induces tumours of the kidney but not of the liver^{1,4}. (Liver tumours are produced in the adult rat only if dimethylnitrosamine is given after partial hepatectomy⁵ or by repeated small doses or prolonged feeding^{1,2}.) Similar experiments with certain nitrosamides have shown that although they penetrate and subsequently react to alkylate cellular components to a similar extent in many tissues, they do not produce an equal incidence of tumours in all these organs^{1–4}. It seems therefore, that different tissues vary quantitatively in their inherent suscep-

tibility to these carcinogens. We report here results which may provide an explanation for this difference and also for the observation that large doses of dimethylnitrosamine are carcinogenic to the kidney while small doses are not.

The major product of the reaction with DNA of metabolites derived from nitrosamines is 7-alkylguanine but other sites are also attacked—alkylation of the O⁶ and 3 positions of guanine, the 3 position of cytosine, the 3 and 7 positions of adenine and the O⁴ position of thymine have been detected^{6–8}. O⁶-Alkylguanine, in particular, has been shown to miscode and is therefore potentially mutagenic^{9,10}.

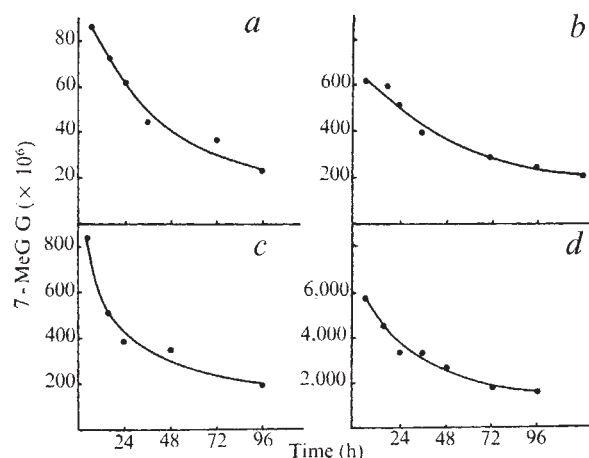


Fig. 1 Formation and subsequent loss of 7-methylguanine (7-MeG) from rat kidneys (a and c) and liver DNA (b and d) after administration of dimethylnitrosamine. Female Wistar rats (160 g) were treated with either 2.5 mg per kg body weight (a and b) or 20 mg per kg body weight (c and d) of ¹⁴C-dimethylnitrosamine (DMN) (specific activity 28 mCi mmol⁻¹ and 4 mCi mmol⁻¹, respectively, prepared as previously described⁴). At various times after the injection, DNA was isolated from kidney and liver⁴, hydrolysed in 0.1 N HCl (refs 7 and 11) and the liberated purines separated by column chromatography on Sephadex G10 or Dowex-50 (refs 7, 8 and 11). The amounts of 7-methylguanine and O⁶-methylguanine present were determined from the radioactivity in the fractions corresponding to the positions of authentic unlabelled markers; the amount of guanine present was determined from the absorbance of the guanine fractions. The results are expressed as the proportion of guanine methylated at the 7-position (7-MeG) to total guanine (G) present.

Further, when a range of alkylating carcinogens of different potency are compared, the ability to form this product correlates much better with the carcinogenicity than the ability to form 7-alkyldeoxyguanosine or the other minor products described above^{3,7,11}. One possible explanation for the greater sensitivity of the kidney than the liver to a large single dose of dimethylnitrosamine would be any difference in the ability of the tissues to repair lesions produced in DNA before cell division. We have therefore studied the persistence of O⁶-methylguanine and 7-methylguanine in DNA of rat liver and kidney after a large dose (20 mg per kg body weight) of dimethylnitrosamine which would produce some kidney tumours and a small dose (2.5 mg kg⁻¹) which would not and have found a marked difference in the rate of removal of O⁶-methylguanine from the kidney DNA. These results may have considerable importance in determining the tissue specificity of tumour production by nitroso compounds and provide further support for the recent hypothesis¹² that the rate of elimination of O⁶-alkylguanine from DNA may be an important factor in neoplastic transformation by alkylating carcinogens.

After a dose of 20 mg kg⁻¹ dimethylnitrosamine, the 7-methylguanine formed in liver and kidney DNA was lost at a rate corresponding to a half life of about 60 h (Fig. 1). A similar rate of loss was found in kidneys of rats treated

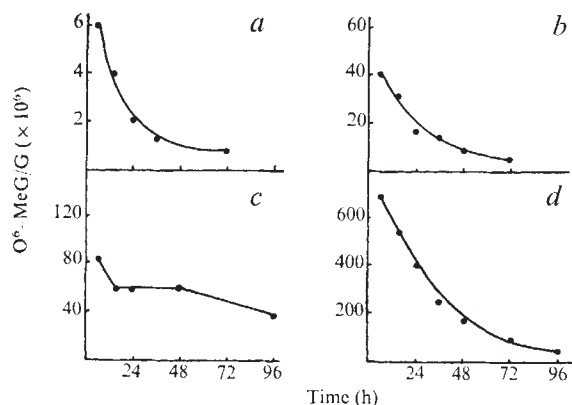


Fig. 2 Formation and subsequent loss of O^6 -methylguanine in rat liver and kidney DNA after administration of dimethylnitrosamine. Proportion of guanine methylated at the O^6 position (O^6 -MeG) to total guanine (G) present in DNA. Experimental procedure as in Fig. 1.

with 2.5 mg kg^{-1} but the rate of loss in the liver was slightly slower after this dose. These data are in reasonable agreement with those published^{8,13-15} for various doses and shows that 7-methylguanine is lost from DNA *in vivo* at a rate somewhat faster than that expected from chemical depurination at neutral pH (ref. 16). The possible contribution to this loss from the necrosis of highly alkylated cells and dilution by new DNA synthesis could not be determined accurately, however, and it is therefore not possible to determine to what extent an enzymic excision mechanism contributes to the removal.

Similar data for the formation and loss of O^6 -methylguanine are shown in Fig. 2. In both tissues shortly after administration of the carcinogen the amount of O^6 -methylguanine formed was only about 10% of the amount of 7-methylguanine. O^6 -methylguanine in DNA is chemically stable at neutral pH (refs 8 and 17) but this product was lost from the DNA of liver after either dose with a half life of about 20 h. This is much faster than the loss of 7-methylguanine and therefore could not have resulted from cell death or DNA replication, which would affect both products equally. Clearly, an enzyme system not yet characterised is responsible for the removal. A similar system must also be present in the kidney, since O^6 -methylguanine was lost from the kidney DNA after the low dose of dimethylnitrosamine at a rate only slightly slower than in the liver. After the larger dose, however, there was a striking difference between the two tissues in the persistence of O^6 -methylguanine in DNA. In the liver (where neither the large nor the small dose is effective in inducing tumours) O^6 -methylguanine was lost from DNA at a similar rate after both doses, but in the kidney after the large dose (which does induce kidney tumours) there was an initial fall of about 30% in the amount of O^6 -methylguanine present in DNA between 6 and 15 h after giving the nitrosamine; but there was practically no decline for 50 h and only a slow rate of loss after this period (Fig. 2).

Thus the potentially mutagenic O^6 -methylguanine is much longer-lived in kidney DNA after a large dose of dimethylnitrosamine than after a smaller dose or in liver DNA after either dose. This correlation with the induction of tumours, together with recent data showing that the brain, which is much more sensitive than the liver to the carcinogenic activity of nitrosamides, is much less active than the liver in catalysing the removal of O^6 -alkylguanine from DNA (refs 12 and 18), provide support for the hypothesis that the differing susceptibilities of organs towards carcinogenic stimuli may be determined by the ability to repair certain alterations produced by the carcinogen in DNA. This

ability seems to vary both from tissue to tissue and with the dose of carcinogen used. It is not yet known why the loss of O^6 -methylguanine from kidney DNA is so much slower after the larger dose of dimethylnitrosamine, but it is possible that the enzyme system responsible for the removal is somehow inhibited by the large dose of the carcinogen or alternatively, has only a limited capacity to remove this product. Further experiments to study these possibilities and characterise the enzymic reactions involved are in progress. This process may involve a similar activity to that recently demonstrated in microorganisms^{17,19}.

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Base changes in the recognition site for *ter* functions in lambdoid phage DNA

THE DNA of bacteriophage λ is a linear duplex of about 45,000 base pairs with single-stranded projections of twelve bases at its 5' termini^{1,2}. These projections are of complementary base sequence³ and result from the action of a λ gene product⁴, *ter*, on a concatenated form of the phage DNA⁵. The base sequences of the single-stranded regions have been determined from repair reactions with DNA polymerase and radioactively labelled nucleoside triphosphates³ and from analyses of DNase digestion products from 5'-terminally labelled DNA⁶. The 3' terminal sequences of λ DNA have also been determined by analysis of DNase digests of DNA labelled at or near its 3' termini in exonuclease-repair reactions with T4 DNA polymerase⁷. Together, these results gave a sequence of 25 base pairs in the region of the DNA molecule at which the *ter* function interacts (*cos*). The sequence between the nicks that the *ter* enzyme would be required to make in the concatenated DNA is bisected by an axis of twofold rotational symmetry. This sequence provided an example of discontinuous or hyphenated symmetry in a DNA sequence recognised by a protein⁷. The base sequences of the *lac* operator⁸, the λ promoter⁹, and the promoter¹⁰ for tRNA^{Tyr}_{sw111} also contain hyphenated elements of symmetry.

Of the sixteen base pairs confined between the limits of the symmetrically disposed base pairs, twelve are symmetrically arranged about the axis. The classical biochemical genetic approach would define the bases essential for recognition by the *ter* system as those that cannot be changed without leading to

loss of function. Mutations of essential bases in the recognition sequence would thus be effectively lethal, whereas mutations of inessential bases could not be detected readily. Comparison of sequences from different lambdoid phages whose *ter* systems could cross react might well provide information equivalent to that from a series of mutants. We therefore analysed the 5' terminal sequences of DNA from several other lambdoid phages⁶, some of which had *ter* systems that were known to cross react¹¹. The DNA from four of these ($\phi 80$, 82, 424 and 21) had terminal sequences identical to those of λ DNA, but we report here the 5' terminal sequences of DNA from another lambdoid phage¹², $\phi D326$, which is closely related to $\phi 80$. The sequences differ in two nucleotides from the equivalent sequences in λ DNA.

The 5' terminal sequences of $\phi D326$ DNA were determined by the method used previously for several phage DNA preparations. DNA was extracted from the purified phage by treatment with phenol, dephosphorylated with bacterial alkaline phosphatase, and labelled at its 5' termini with ³²P using the polynucleotide kinase reaction. The labelled DNA was purified and digested with pancreatic DNase and the oligonucleotides fractionated by ionophoresis on AE81 and DE81 ion-exchange papers¹³, and by two-dimensional ionophoresis on cellulose acetate and thin layer chromatography on PEI cellulose¹⁴ (Fig. 1). The base sequence of each radioactive oligonucleotide was determined from ionophoretic analysis of its products on partial digestion with venom phosphodiesterase. The sequences deduced (Table 1) for the cohesive ends of $\phi D326$ DNA are

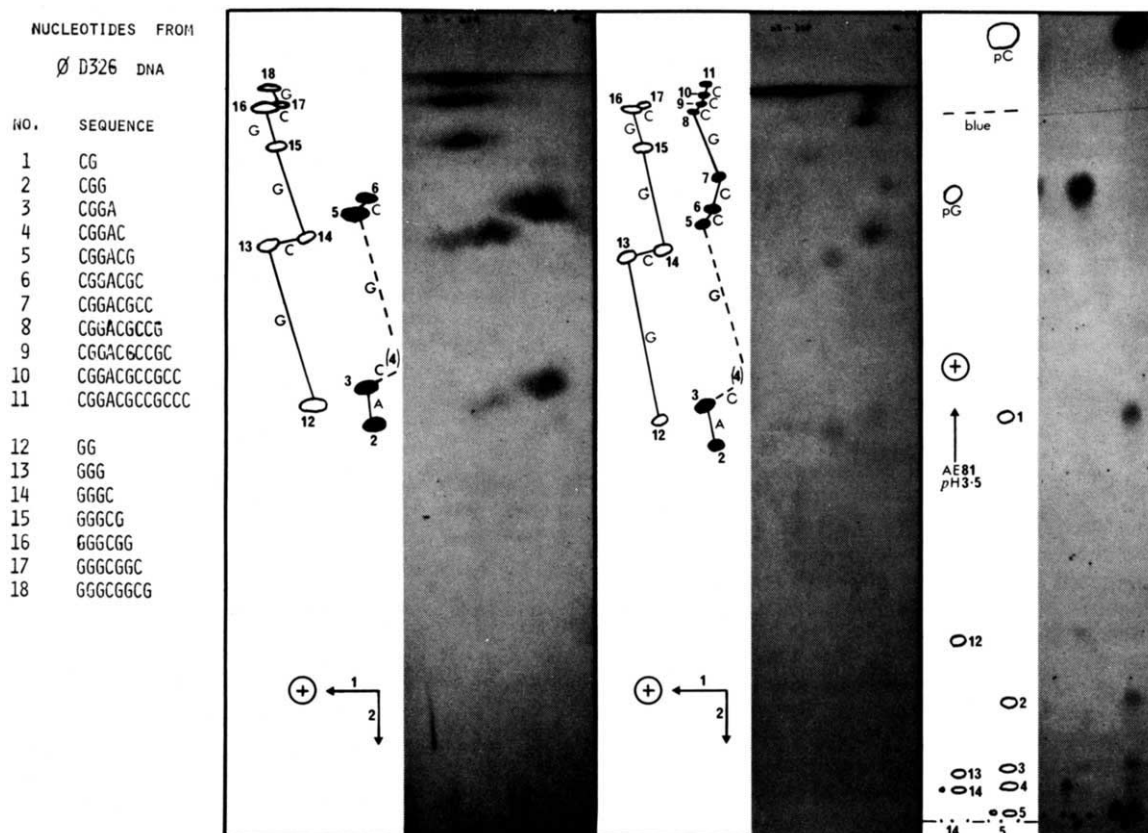


Fig. 1 Nucleotide maps of DNase digests of terminally labelled DNA from $\phi D326$. Stocks of the phage were prepared as described previously¹² and purified by two cycles of equilibrium centrifugation in CsCl (41.5% w/w). DNA was isolated from the dialysed phage preparation by rolling gently with freshly distilled phenol (three times) and was dialysed against four changes of 0.01 M Tris-HCl, pH 8.0, 1 mM EDTA. Terminal phosphate groups were removed with bacterial alkaline phosphatase (Whatman, UK; 25 $\mu\text{g ml}^{-1}$ (2 h at 37 °C)) which was then eliminated by further extraction with phenol and the preparation dialysed against 0.02 M Tris-HCl, pH 7.5. Labelling with polynucleotide kinase from *Escherichia coli* B infected with phage T4 and $\gamma\text{-}^{32}\text{P}$ -ATP (specific activity, 14.8 Ci mmol⁻¹; Radiochemical Centre, Amersham, UK), and purification of the labelled DNA by sedimentation through 6–20% w/v sucrose gradients were carried out as described previously^{6,13}. The DNA (170 μg , 73,000 c.p.m., Cerenkov counts¹⁶) was divided into five portions for digestion with pancreatic DNase in 0.01 M Tris-HCl, pH 7.5, 5 mM MgCl_2 . Two portions were digested at DNase concentrations of 25 $\mu\text{g ml}^{-1}$ (37 °C, 3 h) and the products fractionated by ionophoresis on AE81 paper at pH 3.5 or DE81 paper at pH 2.0 to give oligonucleotides 1–5 and 12–16 the sequences of which were determined from analyses of partial digests with snake venom phosphodiesterase (Table 1). The remaining three portions of the terminally labelled DNA were digested with the same enzyme under milder conditions (2–10 μg DNase ml^{-1} ; 60–90 min at 23 °C) and fractionated by ionophoresis on cellulose acetate at pH 3.5 (in 7 M urea) followed by transfer¹⁷ to thin layers of PEI-cellulose (Macherey Nagel AG, Germany) for development in 1.6 M formic acid adjusted to pH 3.6 with pyridine¹⁴. The oligonucleotides were eluted with 30% v/v triethylamine carbonate and allotted to their respective family on the basis of the 5' terminal mononucleotide identified by ionophoresis on AE81 paper, pH 3.5, after complete digestion with venom phosphodiesterase¹³ (0.1 mg ml^{-1} in 0.02 M Tris-HCl, pH 8.5, 37 °C, 1 h). In the drawings accompanying the photographs, oligonucleotides with a 5' G terminus are shown as open rings and those with a 5' C terminus as filled rings. The sequence of oligonucleotides 2–6 and 12–16 was inferred from their behaviour on the two-dimensional system and confirmed by ionophoretic analysis of partial digests with venom phosphodiesterase (Table 1) on AE81 paper at pH 3.5; examples of these on oligonucleotides 5 and 14 are included on the right of the figure. (Oligonucleotide 4 was obtained from the DNase digests fractionated by ionophoresis on AE81 and DE81 papers and was found in partial digests of oligonucleotides 5 to 9 with venom phosphodiesterase, but it was not found in the milder DNase digests analysed two-dimensionally. Its position on the two-dimensional nucleotide maps, shown in parentheses, has therefore been estimated from the difference in base sequence of nucleotides 3 and 5 and the effects on ionophoretic and chromatographic behaviour resulting from addition of C and G residues to oligonucleotides generally^{18,19}.) The sequence of the longer oligonucleotides was deduced from their behaviour on the two-dimensional separation system, as changes in position are diagnostic of the nucleotide by which two immediate homologues differ^{6,18,19} (these are indicated alongside the lines joining the oligonucleotides from the two 5' terminal families in the drawings to the left of the photographs) and from the necessary complementarity of the two terminal sequences. The different extents of digestion of the two termini of the DNA in the samples shown above is probably fortuitous.

Table 1 Analysis of nucleotides separated by two-dimensional ionophoresis and thin-layer chromatography

Nucleotide no.	Mobility AE	DE	5' Terminus	Products from partial digestion with venom phosphodiesterase				Base sequence of oligonucleotide
1	0.57		C					CG
2	0.16	0.80	C	CG,				CGG
3	0.070	0.57	C	CG,	CGG			CGGA
4	0.044		C	CG,	CGG,	CGGA		CGGAC
5	0.012		C	CG,	CGG,	CGGA, CGGAC		CGGACG
6	0.007		C		CGG,	CGGA, CGGAC, CGGACG		CGGACGC
12	0.26	0.82	G					GG
13	0.066	0.26	G	GG				GGG
14	0.045	0.25	G	GG,	GGG			GGGC
15	0.012		G	GG,	GGG,	GGGC		GGGCG
16	0.003		G	GG,	GGG,	GGGC, GGGCG		GGGCGG

Oligonucleotides have been grouped to show the families of progressively overlapping sequences from each 5' terminus of the DNA. Their number refers to their identity in Fig. 1. Mobilities on AE81 paper (pH 3.5) and on DE81 paper (pH 2.0) are expressed as ratios of the distance moved by the oligonucleotide to that of the blue dye, xylene cyanol FF, and in most cases are the average from several independent experiments. The sequence of each oligonucleotide was deduced from its mobilities and the M values^{13,15} of the products of partial digestion with snake venom phosphodiesterase, when each oligonucleotide gave the successively smaller members of its family (Fig. 1). Partial digests of the oligonucleotides (0.01 mg ml⁻¹ venom phosphodiesterase in 0.02 M Tris-HCl, pH 8.5, 20 µl; 23 °C, 20 min and 60 min) were analysed by ionophoresis on AE81 paper at pH 3.5 or DE81 paper at pH 2.0 (ref. 13).

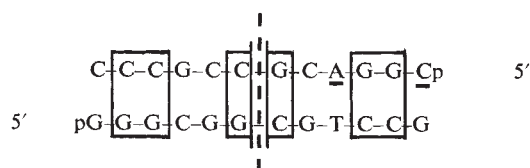


Fig. 2 The 5' terminal sequences of ϕ D326 DNA. The longest oligonucleotide sequences deduced (Fig. 1) have been base-paired and extended by addition of the complementary bases to show the double-stranded structure that would be formed by cohesion of the two single-stranded ends.

written as a base-paired structure in Fig. 2. This structure, like that of the cohesive ends of λ DNA possesses a twofold rotational symmetry; the symmetrically disposed bases are shown enclosed in boxes. The two bases in which this sequence differs from the corresponding region of λ DNA are underlined and both changes are in non-symmetrical positions.

The difference between terminal nucleotide sequences of ϕ D326 DNA and λ DNA could reflect a different sequence requirement for the ϕ D326 *ter* system, or it could indicate that the bases in these positions are unimportant for the *ter* interaction. If the ϕ D326 *ter* system can recognise the appro-

priate nucleotide sequence in λ DNA it should excise λ from a tandem dilyso-gen. To test this, we followed the genetic approach by which Mousset and Thomas demonstrated the existence of the λ *ter* system.

RecA strains lysogenic for $\lambda imm^h A^-$ and $\lambda imm^{434} R^-$ (strains *a* and *b* in Fig. 3) were constructed. On the prophage map the *ter* recognition sequence, *cos*, is between the λ genes *R* and *A* and the order of the prophages is defined by the finding that superinfection with the heteroimmune phage λimm^{21} excises $\lambda A^- imm^{434} R^-$ phages from strain (*a*) and $\lambda A^+ imm^h R^+$ phages from strain (*b*) (ref. 4). Since the λ site-specific recombination system, *int*, can excise phage λ from a λ dilyso-gen⁴, the heteroimmune superinfecting phage chosen was an integration defective phage ($\lambda bio1$). Phages ϕ 80 (ref. 23) and ϕ D326 (ref. 12) do not integrate at the λ attachment site and therefore have *int* systems with different specificities from that of λ . Phages ϕ 80 and ϕ D326 have the same *int* specificity (N.E.M., unpublished) and since ϕ 80 *int*⁺ and *int*⁻ phages were found to be

Fig. 3 Structures of the dilyso-genic strains used. The bacterial host was the *recA supII* strain QR48 lysogenised by $\lambda imm^h c1857 Aam32$ and $\lambda imm^{434} Ram54am60$. B.P', P.P' and B'.P are the components of the attachment regions²¹. The markers *A* and *R* are closely linked in the prophage map but are at opposite ends of the vegetative map²². The *ter* function acts at *cos* and breaks the linkage between *A* and *R*.

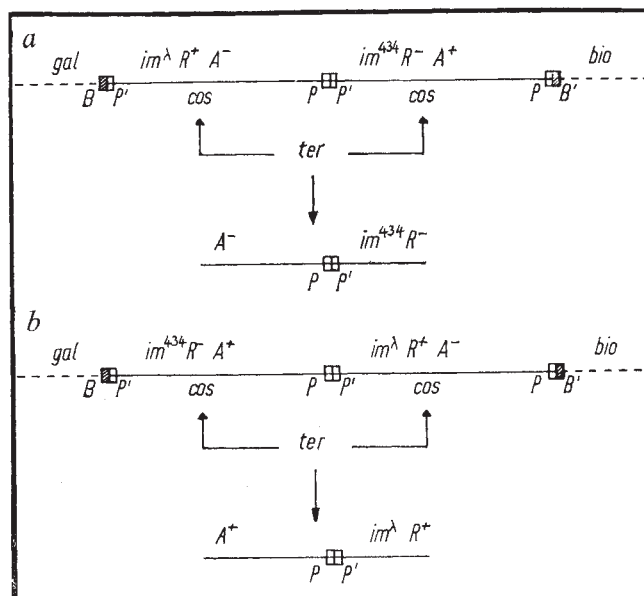
Table 2 Heteroimmune superinfection of the λimm^h-imm^{434} dilyso-gens

Superinfecting phage	Relative yields of phage with immunity of either prophage	
	Strain (<i>a</i>)	Strain (<i>b</i>)
$\lambda imm^{21} bio1$	100*	100†
ϕ 80	46*	61†
ϕ D326	45*	99†
—	1	1

* Over 90% of the phages were $\lambda imm^{434} R^- A^-$

† Over 90% of the phages were $\lambda imm^h R^+ A^+$

The results are the average of two experiments. The dilyso-genic strains were grown at 32 °C and superinfected with heteroimmune phage at a multiplicity of between 3 and 5, as described by Thomas²⁴. Following superinfection with λimm^{21} the lysates were assayed on the *supII* host W1485 ($\lambda imm^{21} Aam32 Ram54am60$) and following superinfection with either ϕ 80 or ϕ D326 on W1485 (ϕ 80). ϕ 80 and ϕ D326 are homoimmune¹². The plates were incubated at 38 °C so that the $\lambda c1857$ plaques (that is λimm^h) were clear and the λimm^{434} plaques turbid. Plaques were purified on the *tonA supII* strain and tested on a *tonA sup*⁰; *tonA* strains are resistant to ϕ 80 (ref. 23) and ϕ D326 (ref. 12). Media and methods were as described previously (ref. 25). ϕ D326 plaques are always variable in size, presumably because this phage adsorbs inefficiently to its host. Since $\lambda imm^{434} R^- A^-$ plaques are extremely small, those excised and therefore packaged by ϕ D326 are very difficult to estimate. This probably explains the relative yield of 45% from strain (*a*) as opposed to 99% from strain (*b*).



equally efficient in excising phage from λ dilysogens, we felt justified in using the efficiencies with which the *int*⁻ phage λ *biolimm*²¹ and the *int*⁺ phages ϕ 80 and ϕ D326 excise phage λ from the two dilysogenic strains as an index of *ter* activity.

The results (Table 2) show clearly that phages λ , ϕ 80 and ϕ D326 can all supply a function able to excise linear chromosomes terminated by genes *A* and *R* from a structure in which these termini are linked. We conclude that the *ter* function of ϕ D326, like that of ϕ 80 (ref. 11), is able to act not only on its own *cos* sites but on those of phage λ , even though in this case the *cos* sequences differ in two base pairs from those of ϕ D326.

Since the ϕ D326 *ter* system is able to recognise the appropriate nucleotide sequence in λ DNA as well as in its own, the nucleotides in which the terminal sequences of these two DNAs differ cannot be essential for physiological function. Both nucleotide changes occur at positions that are not included in the symmetrically disposed locations. The observed results are therefore consistent with the hypothesis that the symmetrically arranged bases constitute the recognition element of the target sequence for the *ter* system, but they do not, of course, prove this.

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Plasmid-determined antibiotic synthesis and resistance in *Streptomyces coelicolor*

In eubacteria, many characteristics¹⁻³ including resistance to antibiotics and other agents are plasmid-determined. In streptomycetes there are indications that plasmid genes are involved in the production of certain antibiotics: kasugamycin and aureothricin by *Streptomyces kasugaensis*⁴, oxytetracycline by *S. rimosus*⁵ and turimycin by *S. hygroscopicus*⁶. An increased proportion of antibiotic non-

producing strains is found after treatment of antibiotic producers with agents known to cause plasmid loss in eubacteria; in *S. rimosus*, crosses provided additional evidence that extrachromosomal genes are involved in antibiotic production. In all three species, the cause of antibiotic non-production, for example whether it involved an interruption of the biosynthetic pathway or a defect in the liberation of antibiotic into the medium, was unknown. We report here that both the production of and resistance to the same antibiotic in *S. coelicolor* A3(2) are determined by genes borne on the plasmid SCP1.

In *S. coelicolor* A3(2) recombination has been used to map more than 100 genes, identified by auxotrophic, resistance and morphological mutations, to a single circular linkage group⁷. Recombination requires cellular contact and leads to the inheritance from each parent of large chromosomal regions; it thus has at least some of the characteristics of conjugation in the Enterobacteriaceae. Moreover, at least part of the exchange of chromosomal markers between mating strains is determined by a plasmid, SCP1. Genetic evidence for the existence of SCP1 is very strong: first,

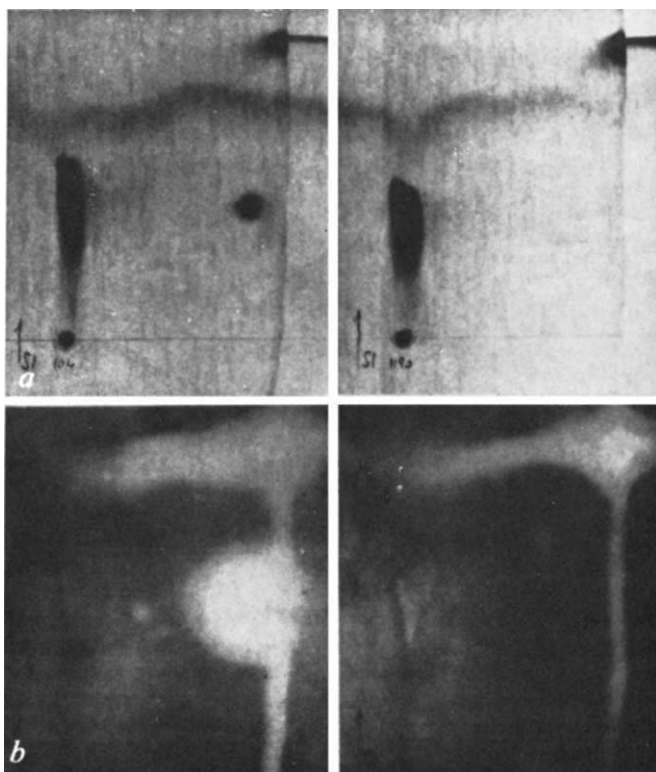


Fig. 1a, Two-dimensional chromatography on silica gel thin-layer plates (Eastman Chromagram No. 13181) impregnated with fluorescent indicator of broth concentrates from cultures of (left) SCP1⁺ strain 104 of *S. coelicolor* A3(2) and (right) SCP⁻ strain 1190. Solvent 1 (run bottom to top in the photograph) was tertiary butyl alcohol-acetic acid-water (72:3:25); Solvent 2 (run left to right in the photograph) was ethyl acetate-acetic acid-water (88:6:6). The chromatograms were photographed in 254 nm ultraviolet light. Note the spot of ultraviolet-absorbing material with *R_f* 0.45 in Solvent 1 (purer material normally runs at 0.75-0.85) and *R_f* 0.85 in Solvent 2 in the SCP1⁺ chromatogram. **b**, Bioautography of the chromatograms shown in **a**. Chromatograms were laid on a basal layer of nutrient agar in a 23 × 23 cm Bio-Assay plate (A/S Nunc, Roskilde, Denmark) and overlaid with molten nutrient agar containing *Bacillus mycoides* (3 ml of late log-phase culture per 100 ml agar) and 0.0012% 2,3,5-triphenyl tetrazolium chloride. The plate was incubated overnight at 36 °C. Note the zone of inhibition of *B. mycoides* by the ultraviolet-absorbing spot in the SCP1⁺ chromatogram (left). Nonspecific inhibition by material accumulating at the solvent fronts is present in both preparations.

strains apparently having the plasmid in the autonomous state ($SCP1^+$) transfer the $SCP1^+$ characteristics independently of chromosomal markers ('infectiously') to $SCP1^-$ strains⁸; second, $SCP1^+$ strains produce $SCP1^-$ strains with frequencies much higher than those expected of mutations⁹, while $SCP1^-$ strains do not revert to $SCP1^+$; third, certain strains, called $SCP1'$ by analogy with F' strains of *Escherichia coli* K12, transfer particular chromosomal genes infectiously, together with the $SCP1^+$ characteristics, to $SCP1^-$ strains¹⁰, as expected if such chromosomal genes have become inserted into the plasmid; fourth, other strains transfer characteristic patterns of chromosomal markers to $SCP1^-$ strains; these are interpretable as having $SCP1$ inserted into the chromosome at various points¹¹.

$SCP1^+$ strains (then called IF) were described as producing a diffusible inhibitor that arrested the development of $SCP1^-$ cultures (called UF); $SCP1^+$ cultures were resistant to this inhibitor⁸. We now show that this material is an antibiotic (rather than, for example, a bacteriocin or bacteriophage) and that several genes controlling its biosynthesis, as well as resistance to it, are borne on the $SCP1$ plasmid.

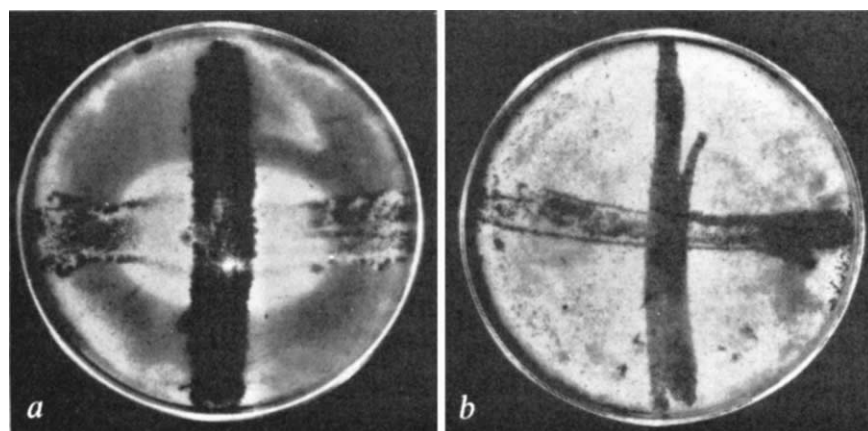


Fig. 2 Tests for antibiotic cosynthesis by pairs of antibiotic-negative mutants. Each plate was prepared by inoculating two mutants, by replica plating from master plates, at right angles on a plate sown with spores of an $SCP1^-$ tester strain. *a*, Cosynthesis by R40 (secretor: vertical streak) and R39 (convertor: horizontal streak): a zone of inhibition of the test strain extends from the centre of the plate along the R39 culture. *b*, Lack of cosynthesis by R40 (vertical streak) and R53 (horizontal streak).

When spores of $SCP1^+$ and $SCP1^-$ strains are inoculated close together on agar, both cultures reach a stage immediately preceding aerial mycelium production before antibiotic diffusing from the $SCP1^+$ culture prevents further development of the nearby $SCP1^-$ culture. Production of antibiotic by the $SCP1^+$ culture, rather than susceptibility of the $SCP1^-$ culture to its action, is correlated with the development of aerial mycelium; young vegetative mycelium of $SCP1^-$ strains is susceptible to antibiotic produced by mature $SCP1^+$ cultures as can be shown by placing agar plugs cut from such cultures on plates inoculated with $SCP1^-$ spores. Susceptibility to the antibiotic is not confined to $SCP1^-$ cultures of *S. coelicolor* A3(2); the antibiotic has a wide antibacterial spectrum. About half of a collection of *Streptomyces* spp. was found to be susceptible¹². Of other actinomycetes and related bacteria, one *Nocardia* sp. was sensitive and two *Mycobacterium* spp. and one *Corynebacterium* sp. were resistant. Members of all Gram-positive eubacterial genera tested were sensitive: *Azotobacter*, *Bacillus* (six species), *Micrococcus*, *Staphylococcus*, *Streptococcus*. One member of each of the Gram-negative genera *Escherichia*, *Klebsiella*, *Salmonella*, *Serratia*, *Agrobacterium*, *Pseudomonas* and *Rhizobium* was resistant, but *Erwinia carotovora* and *Proteus vulgaris* were susceptible. Fungi of the genera *Saccharomyces* and *Penicillium* were unaffected.

The antibiotic is of comparatively low molecular weight,

since it diffuses through dialysis membranes. Two-dimensional chromatography of extracts of culture fluids reveals a single difference between isogenic $SCP1^+$ and $SCP1^-$ cultures: the presence, in $SCP1^+$ preparations, of a discrete ultraviolet-absorbing spot (Fig. 1*a*) which produces a zone of inhibition of the test organism in bioautography (Fig. 1*b*). Antibiotic eluted from this spot inhibited sensitive organisms at concentrations less than $10 \mu\text{g ml}^{-1}$.

Seven strains carrying $SCP1$ -linked mutations resulting in lack of antibiotic activity were isolated from a strain bearing the $SCP1^-cysB^+$ plasmid¹⁰. They were detected by replica plating colonies to plates spread with $SCP1^-$ spores and occurred, after mutagenesis by ultraviolet (254 nm), or by 365 nm light in the presence of 8-methoxypsoralen⁷, with a frequency of about 5×10^{-4} . All the mutants retained antibiotic resistance. Most were revertible to antibiotic production, suggesting that they were point mutations. Plasmid linkage of the mutations was demonstrated by transferring the plasmid from each mutant to a $cysB^-$ $SCP1^-$ strain, which remained antibiotic negative, while acquiring antibiotic resistance and the extrachromosomal $cysB^+$ allele.

Pairs of mutants were tested for antibiotic cosynthesis¹³ by inoculating them at right angles on plates sown with spores of an $SCP1^-$ tester strain. Positive tests (Fig. 2*a*) indicated secretion of a material without antibiotic activity by one strain and its conversion to antibiotic by the other; the shape of the inhibition zone allowed identification of the secretor and convertor mutants. The simplest explanation is that the mutations are in plasmid-linked structural genes controlling a pathway of antibiotic synthesis, rather than genes indirectly concerned in antibiotic production. Four plasmid-coded biosynthetic steps are indicated by the classification of the seven mutants into four cosynthetic classes; doubtless this is an underestimate of the number of steps in the pathway, although some steps yet to be revealed by mutants may be controlled by chromosomal genes.

The mechanism of $SCP1$ -coded antibiotic resistance is unknown; however, the finding that a *Streptomyces* plasmid codes for synthesis of and resistance to the same antibiotic is interesting in relation to the possible origin of plasmid-borne genes conferring resistance to actinomycete antibiotics in Gram-negative eubacteria. The theory that such genes originated in antibiotic-producing actinomycetes, many of which possess antibiotic-inactivating enzymes analogous with those of plasmid-carrying Gram-negative eubacteria¹⁴, does not require such enzymes to be coded for by plasmids in the producing organisms. But transfer to other genera might have been aided if this were the case, a

possibility made more likely by our results with *S. coelicolor*.

A second implication of these results is in the evaluation of techniques of gene amplification and other aspects of plasmid manipulation in developing new strains of industrial antibiotic-producing actinomycetes.

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The β_2 -microglobulin gene is on chromosome 15 and not in the HL-A region

POSSIBLE evolutionary homology between the genetic regions controlling histocompatibility antigens (such as HL-A in man and H-2 in the mouse) and immunoglobulins has been proposed^{1,2}. Recent studies involving β_2 -microglobulin (β_2 m) seem to support this idea. Human β_2 m was found originally in the urine of patients with renal tubular dysfunction. It is also present in serum and on the surface of most types of cell^{3,4}. It has a molecular weight of about 12,000 and shows substantial sequence homology with the constant region domains of immunoglobulin heavy chains^{5,6}. Partially purified papain^{7,8} and detergent⁹ solubilised HL-A molecules, and H-2 molecules¹⁰, consist of two chains one of which is invariant and has been identified as β_2 m¹¹⁻¹³. Chance association during the isolation procedure has been shown to be unlikely by the cocapping on the cell surface of β_2 m with the allogeneic chain of HL-A that carries the usual serologically detected determinants¹⁴⁻¹⁶.

The genes controlling the HL-A determinants form part of a complex genetic region associated with immune functions including transplantation rejection, mixed lymphocyte culture stimulation, graft *versus* host response, cell mediated lympholysis, immune response and some complement functions (see ref. 17). The possibility that all these functions, which involve the cell surface and its role in recognition, are mediated by molecules evolutionarily related to immunoglobulins might be supported by the location of the gene for β_2 m in this region. Using the techniques of somatic cell genetics with human-mouse hybrid cells we have, however, shown that the gene for β_2 m is not located in the HL-A region.

Antiserum to human β_2 m produced in rabbits (Dakopatts, Denmark) was active in a cytotoxicity assay performed using our standard procedure¹⁸. To remove nonspecific toxicity against mouse cells specially selected rabbit complement was absorbed with the 8-azaguanine L cell derivative 1R, which was the parent mouse cell in most of the hybrids tested.

The last dilution of the antiserum which gave a 50% kill in complement-dependent cytotoxicity on primary human fibroblasts was 1/64. The antiserum did not kill the parent

Table 1 Segregation of β_2 m and other human markers in a series of human-mouse hybrids

Hybrid	β_2 m	Chromosome 15* MPI/PK	Chromosome 11*† SA-1	Chromosome X‡ G6PD	Karyotyping carried out
1W1§	—	—	±	+	Yes
2W1§	—	—	+	+	Yes
4W10§	—	—	+	+	Yes
Subclones¶					
G1, G6, G10	—	—	+	—	Yes
4W7§	—	—	+	+	No
Subclone					
R18	—	—	+	—	No
P7A‡‡	—	ND	—	—	Yes
4 12 Z§	—	—	+	+	No
HORL 1,2 and 9§††	+	+	—	+	Yes
HORL 8	+	+	—	+	No
HORL 11	+	+	+	+	No
HORP§††	+	+	+	+	No
Subclones					
24, 25	+	+	+	+	Yes
14	—	—	+	+	Yes
27R	+	+	+	—	Yes
3W4§	+	+	+	+	No
Subclone**					
DF42	+	+	+	—	No
DUR¶¶	+	+	+	+	Yes
4, 5, 10	+	+	+	+	Yes
DUR¶¶	—	—	+	—	Yes
4R, 5R, 10R	—	—	+	—	Yes
AD§§	—	+	+	+	Yes

* Chromosome 15 markers assayed for as described ref. 23.

—, Absence; +, presence in a majority of cells; ND, Not done; ±, presence in a small proportion of cells.

† Chromosome 11 linked antigen SA-1 as described in ref. 30, LDHA as described in ref. 31. Either was taken to indicate the presence of chromosome 11.

‡ Chromosome X. G6PD assayed for as described in ref. 32.

§ Hybrids between normal peripheral blood lymphocytes and 1R. See refs 31, 32 and 33.

¶ 8-Azaguanine selected derivatives of 4W10.

|| 8-Azaguanine selected derivatives of 4W7.

** 8-Azaguanine selected derivatives of 3W4.

†† HORL and HOPR hybrids and subclones are described in detail in ref. 23.

‡‡ Described in ref. 34.

§§ See text and ref. 25.

¶¶ The DUR hybrids were made between 1R and a human X/15 translocation fibroblast in which the X/15 chromosome is active. Selection in 8-azaguanine resulted in the loss of both chromosome X and 15 markers. Further details of the analysis of these hybrids will be published elsewhere.

mouse 1R cells but had a titre of 1/32 on a representative positively reacting human-mouse hybrid, HORL9. The specificity of this reaction on hybrid cell lines was checked by absorptions with different cells and with partially purified β_2 m. Absorption with mixed human type A and O red blood cells¹⁹ (three times with an equal volume), 1R (two times with 2×10^8 cells ml⁻¹) or spleen cells taken from CBA and C3H mice, the strain from which L cells are derived (two times with 5×10^8 cells ml⁻¹) failed to reduce the titre of the antiserum on primary human fibroblasts or the hybrid, HORL9. In contrast, the antiserum's activity on either fibroblasts or the hybrid could be totally absorbed by HORL9 (two times 2×10^8 ml⁻¹), the human lymphoblastoid cell line T51 (2×10^8 ml⁻¹) and 100 μ g ml⁻¹ of partially purified β_2 m. The β_2 m (a gift from Professor B. Pernis) was prepared from the urine of crash victims; the material showed five equally staining bands by SDS electrophoresis. A more highly purified preparation prepared from urine by D. Snary and M. Crumpton, showing only one major band and two minor bands on SDS electrophoresis, also completely absorbed the antiserum at a concentration of about 20 μ g ml⁻¹. On Ouchterlony double diffusion plates both preparations gave a single precipitin line with the Dakopatts antiserum. The antiserum absorbed with 4×10^8 1R cells ml⁻¹

gave this precipitin line while absorption with 4×10^8 HORL9 cells ml^{-1} removed it. A goat antiserum to human $\beta_2\text{m}$ (Kallestad) gave a reaction of total identity with the Dakopatts antiserum on Ouchterlony plates against partially purified $\beta_2\text{m}$. It also gave identical cytotoxic reactions with the Dakopatts antiserum.

The cytotoxicity reactions were confirmed using immunofluorescence. 1×10^5 test cells were mixed with 50 μl of a suitable dilution (1/50) of test antiserum; after 30 min the cells were washed three times and suspended in 50 μl of a 1/20 dilution of fluorescein-labelled sheep anti-rabbit IgG (Wellcome). The cells were incubated at 4 °C 30 min, washed three times and mounted in 50% glycerol in phosphate buffered saline on a slide under a sealed coverslip. The cells were then scored under a Leitz orthoplan fluorescence microscope fitted with a ploom epiilluminator, a BG12 primary filter, K510 secondary filters and a K495 suppression filter. Species specificity in immunofluorescence was only achieved after absorption with 2×10^8 1R cells ml^{-1} . Further absorption with 1R or human red blood cells failed to remove any activity on human lymphoid cells or the hybrid HORL9. As in cytotoxicity, the reaction in immunofluorescence could be totally absorbed by the human lymphoid cell T51.

The specificity of the anti-human $\beta_2\text{m}$ was further checked by immunoprecipitation of membrane extracts. Incubation²⁰ of chloramine T ¹²⁵I-labelled deoxycholate-solubilised membranes²¹ from a human lymphoblastoid cell line with rabbit anti- $\beta_2\text{m}$, followed by the addition of horse anti-rabbit IgG, could be used to precipitate a low molecular weight protein of the same size as $\beta_2\text{m}$, identified on SDS polyacrylamide gels. A similar precipitation procedure with mouse spleen cell membranes did not yield this protein. It has been suggested that rabbit anti-human $\beta_2\text{m}$ (ref. 22) can recognise mouse lymphocytes, causing B cell mitogenesis. These authors agree with us, however, that the antiserum is not cytotoxic for mouse cells.

The results of testing the anti- $\beta_2\text{m}$ sera by complement-dependent cytotoxicity and immunofluorescence, as described above, on a series of human-mouse hybrids are given in Table 1. The two techniques used gave identical results. Work in our laboratory²³ using human-mouse somatic cell hybrids has provided evidence that the genes for mannose phosphate isomerase (MPI, EC 5.3.1.8) and pyruvate kinase 3 (PK-3, EC 2.7.40) can be assigned to chromosome 15. Our study of these and other hybrids, as well as providing more data on this linkage, provides evidence that $\beta_2\text{m}$ is also coded for by a gene on chromosome 15. The origin of most of the hybrids studied and the techniques for their production, propagation and chromosomal analysis were as previously described (see refs in Table 1 and ref. 24).

The $\beta_2\text{m}$ positive hybrid HORL9 has only three human chromosomes²⁴, X, 11 and 15. HOP27R, on the other hand, is an 8-azaguanine-resistant hybrid derivative which lacks the X chromosome, but is $\beta_2\text{m}$ positive. On the basis of these data, $\beta_2\text{m}$ must be coded for by a gene on either chromosome 11 or 15. HORL8, however, which lacks chromosome 11 and is $\beta_2\text{m}$ positive excludes linkage to chromosome 11. From Table 1 it can be seen that four primary hybrids with chromosome 15 have $\beta_2\text{m}$ and six primary hybrids lacking chromosome 15 lack $\beta_2\text{m}$. The corresponding subclone numbers are 12 (+ +) and 8 (— —). The only exception to the expression of human $\beta_2\text{m}$ in human-mouse hybrids with chromosome 15 is AD, which comes from a cross of the human lymphoblastoid line Daudi by the mouse L cell derivative A9 (ref. 25). This exception agrees with the fact that Daudi does not express $\beta_2\text{m}$ ⁴, which we have confirmed. This suggests that the lack of expression of $\beta_2\text{m}$ in Daudi may be due to a change in the genetic region encompassing the $\beta_2\text{m}$ gene, a possibility which can be checked by analysis of more clones derived from Daudi $\times$ A9 hybrids.

The only simple conclusion consistent with these data is that $\beta_2\text{m}$ is coded for by a gene located on chromosome 15. We have obtained further confirmation of this assignment by the use of anti- $\beta_2\text{m}$ and complement to select for hybrids which have lost the chromosome carrying the $\beta_2\text{m}$ gene.

It should be noted that the fact that HORL9 absorbs out all the activity of the Dakopatts antiserum on human fibroblasts, together with the lack of reactivity of this serum on hybrids containing human chromosomes X and 11 without 15, is strong evidence for the antiserum recognising only a product of chromosome 15, both on human-mouse hybrids and on normal human cells. This product is human $\beta_2\text{m}$ as shown by the absorptions with partially purified $\beta_2\text{m}$.

Population and family studies²⁶ as well as inter²⁷ and intraspecific²⁸ hybrids have been used to assign the complex HL-A region to chromosome 6. The two polypeptides of the HL-A molecule are therefore coded for by unlinked genes. This result does not, of course, necessarily imply that the genes for the allogeneic chain of the HL-A molecule are not related to the $\beta_2\text{m}$ gene. The light and heavy chain genes of the immunoglobulins, which are evolutionarily related, are unlinked²⁹. Our data do, however, raise the question of how many genetic regions exist in the human genome whose evolution can be traced to a common ancestry with the immunoglobulin genes. The possibility that the immunoglobulin heavy chain genes are linked to the $\beta_2\text{m}$ gene on chromosome 15 has not yet been excluded.

It is interesting that none of the hybrids in Table 1 has chromosome 6 nor can they be killed by HL-A antisera in a normal cytotoxicity test. Hybrids derived from HORL9 with chromosome 15 as the only human chromosome present can express $\beta_2\text{m}$, strongly suggesting that human $\beta_2\text{m}$ can be expressed on cells in the absence of the products of the human major histocompatibility region. We are now using our hybrids to study the possible interaction between mouse H-2 allogeneic chains and human $\beta_2\text{m}$. In collaboration with R. Kennett, we have recently detected HL-A on human-mouse hybrids by direct cytotoxicity and absorption in the presence of human $\beta_2\text{m}$.

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bility has been studied in detail by measuring the cytotoxicity of T cells from donors immunised with either lymphocytic choriomeningitis (LCM) or ectromelia virus using target cells infected with the homologous virus.

These two viruses are very different, LCM being a non-cytopathic, single-stranded RNA virus which acquires an envelope by budding from infected cell membranes, whereas ectromelia is a cytopathic DNA virus which is assembled completely within infected cells⁹. Evidence described in detail elsewhere^{4,10,11} indicates that immune T cells kill virus-infected target cells directly by contact without production of detectable soluble factors or any requirement for ancillary cells such as mononuclear phagocytes or thymus-independent (B) cells. Experiments with target cells of different H-2 haplotypes and a repertoire of inbred mouse strains, including congenic pairs, have shown that the non-H-2 genetic background, the M locus and H-2 public specificities are irrelevant^{4,11}. Maximal cytotoxicity only occurs when immune T cells and virus-infected target cells share the same H-2 haplotype^{4,11}.

These findings suggest that the genes required are either the H-2 private specificities, which code for the major H-2 antigens on which the haplotype classification is based, or genes closely

Table 1 H-2 composition of mouse strains used

Strain	H-2 regions					
	<i>K</i>	<i>A</i>	<i>I</i>	<i>B</i>	<i>C</i>	<i>D</i>
B10.A	k	k	k	k	d	d
B10.A(2R)	k	k	k	k	d	b
B10.A(4R)	k	k	b	b	b	b
A.TL	s	k	k	k	k	d
A.TH	s	s	s	s	s	d
C3H.OH	d	d	d	d	d	k
C3H.OL	d	d	d	d	k	k
AQR	q	k	k	d	d	d

linked to them in the *K* and *D* regions of the H-2 complex. Identity of *Ir* genes, which map in the *I* region between *K* and *D* (Table 1) and which are common to many different H-2 haplotypes, should not be sufficient¹². This reasoning was tested directly using mouse strains bearing recombinant H-2 haplotypes (Table 1). Immune spleen cells were obtained from donor mice immunised with either LCM virus or ectromelia virus as

Genes required for cytotoxicity against virus-infected target cells in *K* and *D* regions of H-2 complex

EVIDENCE is mounting that in mice, certain specific immunological effector functions of thymus-derived (T) lymphocytes are efficient only when donors of T cells and the cells with which they interact have at least a part of the H-2 gene complex in common¹⁻⁸. Examples include T helper function *in vivo* and *in vitro*¹⁻³, cytotoxicity mediated by T cells against virus-infected⁴⁻⁶ or TNP-modified⁷ target cells *in vitro*, immunopathology mediated by T cells⁴, or protection against bacterial⁸ and viral infection⁶ *in vivo*. This requirement for H-2 compati-

Table 2 Activity of splenic cytotoxic T cells* from LCM-immunised mice against target cells infected with LCM virus

Mouse strain†	Spleen cell status	L929 targets (H-2 ^k)		P-815 targets (H-2 ^d)		Mouse embryo cells (H-2 ^b)	
		Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
B10.A	Normal	15.9 ± 1.0	19.4 ± 1.3	21.4 ± 1.4	30.1 ± 3.2	36.8 ± 1.6	45.1 ± 1.4
	Immune	17.2 ± 1.7	72.6 ± 0.9	31.2 ± 2.3	86.5 ± 1.2	38.5 ± 2.9	45.9 ± 1.8
B10.A(2R)	Normal	24.9 ± 0.7	20.9 ± 1.8	22.7 ± 1.1	29.0 ± 3.7	40.1 ± 0.7	48.2 ± 0.8
	Immune	21.9 ± 2.0	80.9 ± 2.6	41.7 ± 0.9	49.0 ± 1.3	42.7 ± 2.1	61.9 ± 1.8
B10.A(4R)	Normal	17.0 ± 0.8	18.5 ± 2.1	32.1 ± 3.2	26.0 ± 4.5	39.6 ± 1.0	45.7 ± 2.0
	Immune	18.9 ± 1.3	74.3 ± 1.6	40.7 ± 1.9	35.9 ± 1.7	44.4 ± 1.4	59.7 ± 1.5
Mouse macrophages, SJL (H-2 ^s)							
A.TL	Normal	14.1 ± 0.6	16.5 ± 0.9	14.3 ± 0.9	14.1 ± 1.1	40.7 ± 4.2	39.1 ± 3.7
	Immune	17.6 ± 1.7	17.6 ± 1.7	16.2 ± 1.1	64.4 ± 2.4	39.6 ± 2.4	76.1 ± 2.9
A.TH	Normal	12.3 ± 0.7	13.0 ± 0.9	30.5 ± 4.5	28.6 ± 2.1	ND	ND
	Immune	13.2 ± 1.6	16.3 ± 1.9	30.1 ± 4.8	81.0 ± 1.2	ND	ND
C3H.OH	Normal	20.8 ± 0.6	15.4 ± 1.4	14.1 ± 0.5	27.8 ± 1.1	ND	ND
	Immune	22.4 ± 1.1	56.5 ± 2.0	23.7 ± 0.5	74.5 ± 2.8	ND	ND
C3H.OL	Normal	23.2 ± 0.6	27.4 ± 0.6	15.3 ± 0.4	17.2 ± 0.8	ND	ND
	Immune	52.8 ± 2.2	80.5 ± 1.3	18.0 ± 0.3	49.0 ± 0.8	ND	ND
Mouse macrophages, DBA/1 (H-2 ^q)							
AQR	Normal	ND	ND	16.1 ± 0.5	16.9 ± 0.6	39.1 ± 3.5	36.9 ± 2.6
	Immune	ND	ND	28.0 ± 0.5	92.6 ± 0.6	44.8 ± 2.0	62.6 ± 2.3

*Expressed as percentage ⁵¹Cr released (mean of four replicates ± s. e. m.) over 16 h at a killer-target ratio of 30:1 (corrected for water lysis). Significant specific lysis (*P* < 0.05) is indicated by italics.

†Data given in this table were derived from several separate experiments in which the various mouse strains were tested. CBA.H (H-2^k), BALB/c (H-2^d), C57BL (H-2^b), SJL (H-2^s) and DBA/1 (H-2^q) mice were always included in the experiment as controls where necessary. They gave specific lysis only with H-2-compatible infected target cells (see refs 4 and 9) (data not shown).

ND, not determined.

Table 3 Activity of splenic cytotoxic T cells* from ectromelia-immunised mice against target cells infected with ectromelia virus

Mouse strain†	Spleen cell status	L929 targets (H-2 ^k)		P-815 targets (H-2 ^d)		Mouse embryo cells (H-2 ^b)	
		Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
B10.A	Normal	28.2 ± 0.5	28.3 ± 0.5	17.2 ± 0.6	29.6 ± 0.3	34.7 ± 1.5	34.1 ± 1.0
	Immune	40.5 ± 0.3	95.5 ± 1.3	24.6 ± 1.1	59.4 ± 1.2	34.1 ± 1.6	32.9 ± 1.0
B10.A(2R)	Normal	25.6 ± 2.3	26.7 ± 1.7	22.0 ± 1.5	24.0 ± 1.6	38.1 ± 1.8	34.5 ± 1.7
	Immune	29.7 ± 1.2	83.4 ± 1.9	27.0 ± 1.6	37.8 ± 3.1	41.7 ± 1.2	67.1 ± 1.3
B10.A(4R)	Normal	25.5 ± 1.2	27.4 ± 2.3	28.6 ± 3.0	23.6 ± 0.8	45.5 ± 2.0	34.7 ± 1.1
	Immune	30.1 ± 1.2	78.5 ± 2.7	31.1 ± 4.0	30.5 ± 3.5	40.3 ± 1.9	58.8 ± 0.9
A.TL	Normal	35.6 ± 1.3	31.5 ± 1.4	19.5 ± 0.2	23.7 ± 0.5		ND
	Immune	42.3 ± 1.7	44.0 ± 1.5	32.3 ± 0.7	78.4 ± 0.6		ND
A.TH	Normal	36.1 ± 1.2	38.0 ± 2.1	20.1 ± 0.3	22.1 ± 0.4		ND
	Immune	39.0 ± 0.8	37.4 ± 1.2	21.6 ± 0.5	63.1 ± 1.3		ND
C3H.OH	Normal	21.3 ± 1.5	21.2 ± 1.1	17.4 ± 0.6	27.4 ± 1.2		ND
	Immune	33.2 ± 2.0	47.0 ± 2.2	37.1 ± 0.7	71.5 ± 3.8		ND
C3H.OL	Normal	32.0 ± 2.9	33.1 ± 2.8	20.6 ± 2.6	22.5 ± 2.1		ND
	Immune	27.2 ± 2.1	50.9 ± 1.7	39.0 ± 1.5	48.0 ± 2.8		ND
Mouse macrophages, DBA/1 (H-2 ^a)							
AQR	Normal	25.8 ± 2.2	20.8 ± 1.0	15.4 ± 0.8	20.9 ± 1.4	39.3 ± 1.8	42.5 ± 3.7
	Immune	39.9 ± 2.1	24.8 ± 0.8	31.5 ± 1.2	58.9 ± 4.1	43.4 ± 3.6	65.0 ± 4.4

*Expressed as percentage ⁵¹Cr released (mean of four replicates ± s. e. m.) over 16 h at a killer–target ratio of 60:1 (corrected for water lysis). Significant specific lysis ($P < 0.05$) is indicated by italics.

†Data given in this table were derived from several separate experiments which included control strains listed in the footnote to Table 2. ND, not determined.

described elsewhere^{5,11}, and specific cytotoxicity mediated by T cells was measured by ⁵¹Cr release from virus-infected target cells of various H-2 haplotypes using optimal spleen cell–target cell ratios^{5,11} (Tables 2 and 3). Immune cells almost invariably caused more lysis than normal cells, irrespective of target cell type, but significant specific lysis was defined as occurring only when combinations of immune cells and infected targets gave ⁵¹Cr release which was significantly higher ($P < 0.05$) than all three control combinations, such as immune cells with uninfected targets, or normal cells with either infected or uninfected targets. Comparison of Tables 1 and 2 shows that specific lysis of LCM-infected H-2^k target cells required immune T cell donors to be of H-2^k type only in the *K* or *D* regions of the gene complex. It was not sufficient for all of the *I* and *S* region to be *k* (as in A.TL mice). Lysis of LCM-infected H-2^d or H-2^b targets also required only *D* region homology. The use of H-2^s and H-2^a macrophages as target cells confirmed that *K* region homology was sufficient.

Results obtained with ectromelia virus (Table 3) were essentially similar to the LCM system, with one exception. Lysis of infected H-2^k target cells by ectromelia-immune cells from C3H.OH or C3H.OL mice (which are of H-2^k type in the *D* region) was not as reproducible or powerful as with LCM. Ectromelia provoked a significant response against infected H-2^k targets (Table 3) in only one out of three experiments with C3H.OH, and one out of two experiments with C3H.OL, whereas a significant response always occurred against infected H-2^d targets. Thus major gene(s) active in the LCM system seemed less active in the ectromelia system. The factors responsible for this variation are not known, but *Ir* genes are candidates for further investigation.

With both viruses, B10.A (2R) gave a small but statistically significant response against H-2^d target cells in one out of two experiments (Tables 2 and 3), suggesting that genes outside the *K* or *D* regions (for example, in *IC* or *S* regions in this case) may sometimes exert a minor influence.

In summary, these data support the concept that the major genes required for cytotoxicity mediated by T cells, of virus-infected target cells are located in the *K* or *D* regions of the H-2 complex. In most cases, these genes are sufficient, without the requirement for *I* region homology. Whether *Ir* genes play a secondary, regulatory role remains to be determined. This is consistent with the hypothesis proposed^{4,5} that the H-2-dependent restriction of lysis, mediated by T cells, of virus-infected target cells results from T cell recognition of altered self antigens (possibly H-2 private specificities) on the surfaces of virus-infected cells.

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Evolutionary conservation of H-Y ('male') antigen

THE male specific (H-Y) antigen of mice was discovered with the observation that within certain inbred strains, females reject male skin grafts, whereas skin grafts exchanged between all other sex combinations are accepted¹ (reviewed in ref. 2). It is now established that females sensitised with male skin grafts (or immunised with male spleen cells) produce antibody which is cytotoxic for sperm³ and dissociated male epidermal cells⁴. Using the sperm cytotoxicity test and the mixed haemadsorption-hybrid antibody (MHA.HA) test, we demonstrated earlier⁵ that the H-Y antigen of mice is cross reactive or identical with antigen

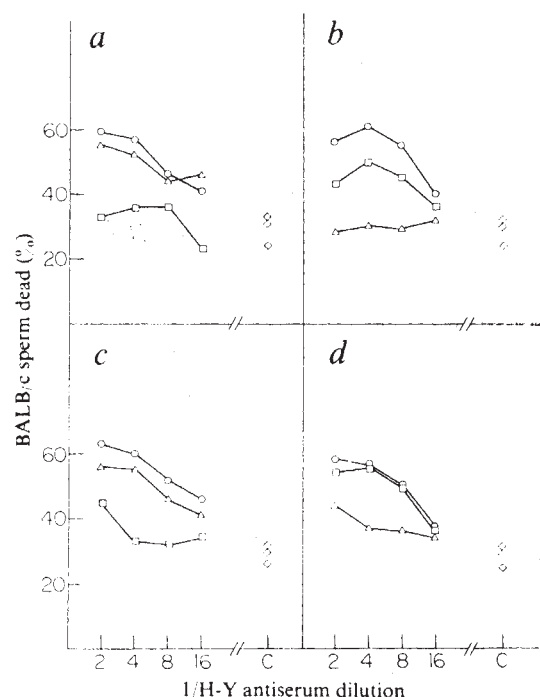


Fig. 1 Summary of cytotoxicity tests on mouse sperm with mouse H-Y antiserum after absorption with male or female cells from four test species. Each point is an average of values from three separate tests (*b*, chicken and *c*, leopard frog), four tests (*a*, mouse) or six tests (*d*, *X. laevis*). $\circ$, Unabsorbed H-Y antiserum; $\triangle$, absorbed with female cells; $\square$, absorbed with male cells (spleen cells of mouse, blood cells of chicken, spleen and liver cells of amphibians); $\diamond$, control, complement included, antiserum omitted. H-Y antisera were prepared in adult C57BL/6 female mice by four to six weekly intraperitoneal injections of $30\text{--}60 \times 10^6$ C57BL/6 male spleen cells. Individual serum samples were tested, diluted one-half and pooled for absorption. Cell suspensions for absorption were prepared as follows. (1) Mouse spleen, according to Wachtel *et al.*⁵. (2) Amphibians: spleen and liver were minced in Earle's balanced salt solution (EBSS) and the supernate, containing free cells in suspension, was removed and centrifuged at $100\text{--}150g$ for 10 min. The cells were washed twice and resuspended in EBSS. (3) Chicken: red blood cells and buffy coat leukocytes were collected from whole heparinised blood after centrifugation at $700\text{--}800g$ 10 min. Cells were washed twice and resuspended in EBSS. All absorptions were carried out on ice for 30–40 min, 1–2 parts serum per 1 part cells. Epididymal sperm, prepared from BALB/c mice according to Goldberg *et al.*³, were incubated with H-Y antisera and with absorbed rabbit serum diluted 1:14 (complement source) for 45–50 min. Trypan blue dye was added during the last 5–10 min of incubation and the tests read in a haemocytometer. Dead sperm stained with the dye.

found in male rats, guinea pigs, rabbits and humans. Since then we have extended our survey to classes other than mammals, and we give evidence here for the occurrence of murine H-Y (or a cross reactive) antigen in the white leghorn chicken (*Gallus domesticus*) and in two amphibian species, the leopard frog (*Rana pipiens*) and the South African clawed frog (*Xenopus laevis*).

H-Y antisera were raised in female C57BL/6 mice by immunisation with male C57BL/6 spleen cells as reported

previously⁵. For each serological test, the selected H-Y antiserum pool was diluted and divided into three parts (as in our earlier studies⁵). The first part was absorbed with female cells of the species being tested (aliquot *A*) and the second, with male cells (aliquot *B*) while the third was not absorbed (aliquot *C*). The three aliquots were then titrated for residual cytotoxic activity against mouse sperm. Absorption of cytotoxic activity from aliquot *A* or *B* would indicate that H-Y antibody must have been removed specifically by female (*A*) or by male (*B*) cells of the species being tested, signifying that the absorbing cells of this species carry a surface component identical or cross-reactive with mouse H-Y antigen.

H-Y antiserum aliquots used in the confirmatory MHA.HA tests were prepared and absorbed in the same way. The indicator cells, mouse sperm, were then incubated with the three aliquots *A*, *B* and *C*, washed, and subsequently exposed to a rabbit hybrid antibody with the specificity: anti-mouse Ig/anti-sheep red blood cells (SRBC). The sperm were again washed and then reacted with SRBC. In this assay, sperm which have taken up H-Y antibody, and therefore also the hybrid antibody, bind SRBC to form rosettes. Absorption of H-Y antibody from

Table 2 Summary of MHA.HA test data: reaction of mouse H-Y antiserum with mouse sperm after absorption with cells from male or female chicken or *X. laevis*

	Serum aliquot		
	<i>A</i> (absorbed ♀)	<i>B</i> (absorbed ♂)	<i>C</i> (unabsorbed)
Chicken	14.2*	23.8	25.4
<i>X. laevis</i>	7.0	15.9	16.6

To avoid repeated centrifugation, the MHA.HA reactions were carried out by centrifuging the sperm through a discontinuous density gradient containing the reagents and wash layers in sequence^{5,18}. Gradients were established in tubes made from 1-ml disposable glass pipettes by addition of heat-inactivated, gamma globulin-free foetal bovine serum (FBS). The concentration of FBS in each layer was adjusted to prevent intermixing. Sperm were obtained from the epididymis of BALB/c mice according to Goldberg *et al.*³. A sample of 0.05 ml of a suspension containing $5\text{--}10 \times 10^6$ sperm per ml was reacted with 0.05 ml H-Y antiserum diluted one-half; the suspension of sensitised sperm was then placed in the gradient tube and centrifuged at $35\text{--}50g$ for 10 min and at $150\text{--}200g$ for 10 min. The pellet (containing sperm and SRBC) was removed and recentrifuged ($35\text{--}50g$ for 10 min) to enhance rosette formation. After standing for 30 min on ice, the pellet was resuspended and samples were read in a haemocytometer. Three tests each (chicken and *X. laevis*) were scored as coded samples by an observer who recorded the counts of rosettes and free sperm. The values (percentages) shown here were derived from the formula: number of rosettes / number of rosettes + free sperm. Any sperm cell to which three or more sheep red blood cells had adsorbed was scored as a rosette.

* Average number of rosettes per 100 sperm cells.

aliquot *A* or *B* in the MHA.HA test is indicated by a decrease in the number of rosettes in comparison with the number observed with unabsorbed antiserum (aliquot *C*).

In view of existing evidence^{6,7} for a sex-associated transplantation antigen in the heterogametic sex of the chicken (which is the female in this case, Table 1) we studied this species first, using both the cytotoxicity and MHA.HA tests. The results of our experiments (Fig. 1, Table 2) indicate that the chicken has an H-Y (H-W) antigen identical or cross reactive with mouse H-Y, and that it is expressed by the female. The decrease in cytotoxicity titre produced by absorption with male chicken cells (Fig. 1) is not more than what can be attributed to anti-complementary effects of the absorption procedure. Such a decrease was not seen in the MHA.HA test, which does not depend on complement (Table 2).

Since birds and mammals represent widely divergent pathways of reptilian evolution, we suspected at this point

Table 1 Sex association of H-Y antigen

Class	Species	Heterogametic sex	H-Y antigen found in
Mammalia	<i>Mus musculus</i> *	Male	Male
Aves	<i>Gallus domesticus</i>	Female	Female
Amphibia	<i>Rana pipiens</i>	Male	Male
	<i>Xenopus laevis</i>	Female	Female

* Mouse

that H-Y antigen would be found in reptiles, and so we next investigated its possible occurrence in Amphibia, a more primitive class of vertebrates. In *R. pipiens* the male is heterogametic as in mammals whereas in *X. laevis* the female is heterogametic as in birds (Table 1). Our serological tests (Fig. 1 and Table 2) demonstrate that cells from both these species contain a surface component identical or cross reactive with H-Y antigen of the mouse. Moreover, the antigen is found in the heterogametic sex of both these amphibian species. Thus it would seem that the cell surface component conferring H-Y antigenicity in the mouse has been conserved through some 300 Myr of evolution (spanning the Carboniferous radiation of amphibians and the Pleistocene emergence of man).

Although the phylogenetic conservation of a particular polypeptide sequence (indicated here by common antigenicity) suggests some essential or highly advantageous role, the reason for the conservation of H-Y antigen is not at all obvious. Nevertheless the association of H-Y antigen with either one sex or the other in those species tested so far seems to indicate a sex-related function of some kind, perhaps one concerned with the recognition of one sex by the other in nature (see discussion by Thomas⁸).

As to whether or not H-Y antigen expression is hormone dependent⁹, several experiments involving male $\leftrightarrow$ female transplantation and/or hormonal manipulation have given conflicting results¹⁰⁻¹⁶. Sex-limited, hormone-dependent traits affecting the cell surface are known however. Indeed the 'Hi' red blood cell agglutinin of chickens is normally confined to females although this trait can be induced in males by treatment with diethylstilboestrol¹⁷. Thus it seems wise to leave open the question of the extent to which expression of H-Y antigen may be influenced quantitatively by sex hormones, although our finding that H-Y may occur in females implies that its expression is not qualitatively hormone-dependent. In this context it remains to be determined whether or not the H-Y structural locus is situated on the Y chromosome. An exceptional species in which H-Y antigen were found in the homogametic sex would suggest that the H-Y structural locus is autosomal (or even X-linked). We are now investigating what may prove to be such an exception.

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Production of lymphoid tumours in hamsters by direct implantation of normal human leukocytes

LEUKOCYTES from patients with various lymphoproliferative diseases can be serially transplanted as malignant lymphoid tumours in immunosuppressed newborn hamsters¹. We report that normal human leukocytes can also be transplanted into newborn hamsters treated with antilymphocyte serum.

Three human leukocyte samples were used; one from peripheral blood of an Epstein-Barr virus (EBV) antibody-positive normal male and two from umbilical cord blood with and without EBV infection. Leukocytes were separated by Dextran sedimentation from 20 ml of peripheral or cord blood and $0.5-1 \times 10^7$ viable leukocytes were implanted intraperitoneally into Syrian golden hamsters less than 24 h of age. This was followed by twice weekly intraperitoneal inoculation of 0.1 ml rabbit antilymphocyte serum against hamster thymocytes. The leukocytes from cord blood were incubated with 1 ml of a filtered freeze-thaw extract of the (EBV+) B95 cell line² at 37 °C for 2 h, before implantation.

All three hamsters transplanted with peripheral leukocytes and two out of three hamsters transplanted with cord leukocytes infected with EBV were found to have lymphoid tumours involving the lymph nodes, liver, lungs and kidneys, when killed on days 11-21. No tumours were observed, however, in hamsters transplanted with cord leukocytes not infected with EBV.

Enlarged inguinal lymph nodes resulting from transplantation of peripheral leukocytes or EBV-infected cord leukocytes were cultured in medium RPMI 1640 supplemented with 20% foetal calf serum, and two lymphoblastoid cell lines were established from a hamster of each group. The cells began to grow vigorously in about 3-4 weeks and have been maintained for 6 months. These cell lines have a normal diploid human chromosome constitution and are positive for EBV nuclear antigen³. Electron microscopy revealed EBV particles in lymphoid cells.

Our observation seems to be analogous with the establishment of lymphoblastoid cell lines *in vitro* from normal human peripheral and cord leukocytes, for which an essential role of EBV has been demonstrated^{4,5}. Successful heterotransplantation of normal human leukocytes into hamsters may be based on the same mechanism as in the *in vitro* system. It is possible that normal human lymphocytes can be transformed *in vivo* by EBV and grow progressively in the immunosuppressed heterologous hosts.

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matters arising

Variation of G

DEARBORN and Schramm have considered limits on the variation of G imposed by the existence of clusters of galaxies and globular clusters of stars¹. Their argument is that if G has been varying too rapidly the clusters would have dispersed and we would not see them now. They conclude that my theory of the variation of G , based on the Large Numbers Hypothesis, is untenable.

These authors (and several other workers in the field) have misunderstood the main feature of my theory. They work with a Newtonian theory in which gravitational mass can vary independently of inertial mass, so they are adopting a pre-Einstein view of gravitation. On such a basis there would be no explanation for the motion of the perihelion of Mercury.

The coefficient G has dimensions and its value depends on what units one uses. One might refer it to standard units of physics, g, s and cm. Then the question whether G varies depends on how these units are defined and is not a fundamental question of physics. One should avoid man-made units and use only those provided by nature. One may take units of time and distance provided by atomic clocks and the velocity of light, and a unit of mass provided by an atomic particle, say the proton. Referred to such units the variation of G has an absolute meaning.

If this G varies, the question arises of how to fit it into physical theory without destroying the successes of the Einstein theory. A natural way of doing that is to suppose that all the laws of classical mechanics, including the Einstein theory, are applicable only when referred to suitable units that differ from the atomic units. This is a development of an idea originally proposed by Milne², that there are two scales of time that are important in physics.

Let us call the units that must be used for the laws of classical mechanics to apply 'mechanical units'. For any problem involving dynamical motions and not referring to atomic processes one may work with mechanical units, and the calculations will then not be affected by the variation of G referred to atomic units. This variation may be ascribed to the variation of the atomic units referred to the mechanical units. Thus calculations of the stability of clusters

can throw no light on the variation of G .

One can obtain evidence about the variation of G referred to atomic units by making astronomical observations with atomic apparatus. One method is to observe accurately the times of lunar occultations with atomic clocks. This method has been used by Van Flandern³ who has obtained evidence that G does vary. Another method is to work with I. I. Shapiro's observations of radar reflected by the planets. These are not yet sufficiently accurate to give a definite result, but one can hope that they will be in the near future.

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Future impact of fossil CO₂ on the sea

WHITFIELD¹ has challenged, on chemical grounds, the conclusion²⁻⁴ that uptake of fossil CO₂ by the sea will eventually lead to unsaturation of calcium carbonate. The optimistic conclusion that "serious environmental effects are not likely in the foreseeable future" is unwarranted for the following reasons. (1) Whitfield's theoretical analysis obviously fails to maintain equivalence between anions and cations as CO₂ is taken up by seawater. This impossible situation means only that the increase in total CO₂ predicted by mixing models is incompatible with the chemistry; changes in the degree of calcium carbonate supersaturation with increasing CO₂ partial pressure (P_{CO_2}) requires further analysis. (2) Whitfield assumed too high an initial concentration of total inorganic carbon (ΣCO_2) in seawater, equivalent to 2.66×10^{-3} M. We have measured ΣCO_2 in hundreds of samples of surface seawater (ref. 5 and A.W.F., and J.L.E., unpublished observations) as have Li *et al.*⁶, which generally falls in the range $2.05 \pm 0.05 \times 10^{-3}$ M. For an initial seawater ΣCO_2 of 2.05 (pH 8.00) and using MacIntyre's⁷ values of the stoichiometric solubility product constants (K_{sp}) of calcite ($10^{-6.17}$) and

aragonite ($10^{-5.97}$), the carbonate concentrations for aragonite and calcite saturation are, respectively, 1.07×10^{-4} M and 0.68×10^{-4} M ($Ca^{2+} = 10^{-2}$ M); the corresponding reductions in carbonate are 0.99×10^{-4} M and 1.38×10^{-4} M, respectively. The latter is only 60% of the value estimated by Whitfield for his higher ΣCO_2 for seawater. (3) The degree of present-day supersaturation of seawater with respect to aragonite and calcite, and how this will change with increasing P_{CO_2} , depend on the choice of the various equilibrium constants of the ionic equilibria involved, about which there is some lack of consensus. The degree of supersaturation is expressed by the ratio $\alpha Ca^{2+} \cdot \alpha CO_3^{2-} / K_{sp}$, where K_{sp} is the thermodynamic solubility product constant of CaCO₃, the α s are ionic activities γM , (where γ is the ion activity coefficient). Berner⁸ measured γCa^{2+} to be around 0.21 ± 0.01 , and γCO_3^{2-} to be around 0.022 ± 0.004 , depending on the chlorinity, in reasonably good agreement with Garrels and Thompson⁹. The very low activity coefficient of CO₃²⁻ ion reflects the fact that most of the carbonate ion in seawater is complexed with various cations. Using K_{sp} values from Latimer¹⁰ and Jamieson¹¹ (K_{sp} at 25°C = $10^{-8.24}$ for calcite, $10^{-8.15}$ for aragonite), Berner calculated the degree of supersaturation for a typical warm (25°C) surface seawater to be 2.8 for calcite and 1.8 for aragonite. On the other hand Li *et al.*⁶ conclude on the basis of their measurements and MacIntyre's values of K_{sp} that the degree of supersaturation of calcite and aragonite in seawater is about 5.5 for calcite and 3.5 for aragonite. There is no question that surface seawater is supersaturated with respect to both minerals at the present time, but there is some uncertainty as to the degree. (4) The solubility of CO₂ and CaCO₃ (both aragonite and calcite) in seawater increase with decrease of temperature. Therefore one needs to be concerned not only with what may happen in warm, tropical seas (at about 25°C) but also the subtropical ocean which Whitfield did not consider. At mid to high latitudes the average sea temperatures are much lower and these areas would be the first to achieve undersaturation of aragonite with increase of P_{CO_2} . Fortunately, the majority of calcareous organisms which flourish in the cold, subtropical seas are calcitic, and therefore less soluble; but aragonitic forms do occur¹². We have

observed (our unpublished observations) that surface seawater equilibrated at 10 °C with CO₂ at $P_{\text{CO}_2} = 1,000$ p.p.m. is unsaturated with respect to aragonite; aragonite dissolves under those conditions. One may therefore ask whether an atmospheric concentration of CO₂ three times the present value is likely to result from future fossil CO₂ production. Zimen and Altenhein⁴ estimated that P_{CO_2} values of 1,000 p.p.m. will occur by about the middle of the next century. We believe an atmospheric level of 1,000 p.p.m. will occur sooner as Zimen and Altenhein's estimate is based on a box model in which CO₂ uptake by the sea is not restricted by the chemistry. Our experiments show that ΣCO_2 of seawater increases much more slowly than the box model allows and thus the sea will not absorb as much CO₂ as Zimen and Altenhein calculated. Consequently, P_{CO_2} will increase faster than predicted by their model. The ocean's ability to absorb the anticipated future fossil CO₂ emissions is so limited that it is not so much a question of whether a level of 1,000 p.p.m. can be reached as to when it will be reached. (6) Elsewhere, Whitfield¹³ re-examined the problem of CaCO₃ unsaturation as a function of P_{CO_2} and temperature. He concluded that aragonite unsaturation can be expected to occur in subtropical seas (average temperature about 10 °C) before the middle of the next century. His theoretical result is in good agreement with our experimental observations. We conclude therefore that there are grounds for serious concern about the impact of fossil CO₂ emissions of the ocean.

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dict the rate of fossil CO₂ accumulation in the oceanic mixed layer. They emphasise¹, as I have done elsewhere^{7,8}, that the calculations² at 25 °C result in an increase in the carbonate alkalinity (CA) ($\text{CA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}]$) which, as they conclude, indicates "that the increase in total CO₂ predicted by the mixing models (refs 3–6) is incompatible with the chemistry". The calculations^{2,6} indeed provide strong grounds for rejecting the mixing models of Fairhall^{5,6} and of Zimen and Altenhein^{3,4} which predict gross accumulations of fossil CO₂ in the oceanic mixed layer. I am glad that there is now some agreement on this point.

The high value of ΣCO_2 that I used does not materially affect my conclusions. Analyses of seawater from Plymouth Sound, using the titrimetric procedure described by Edmond⁹, gave ΣCO_2 values that are comparable with those reported¹ (Table 1). The lower ΣCO_2 values, however, do not necessarily imply lower values of $[\text{CO}_3^{2-}]$. The carbonate alkalinity is a much better diagnostic criterion than ΣCO_2 as it also incorporates the effects of pH on the CO₂ system⁷. It is possible to calculate the partial pressure of carbon dioxide required to reduce these samples to saturation level with respect to calcite or aragonite and also the pH at saturation level using the procedures described earlier (ref. 2, equations (4), (6) and (7) and ref. 7, equation (6)). MacIntyre's original value¹⁰ ($10^{-6.26}$) was used for the solubility product of calcite at 25 °C and 35‰ salinity rather than the higher value ($10^{-6.17}$) quoted by Fairhall and Erickson¹. Similar values

have resulted from the recalculations of MacIntyre's data by Ben-Yaakov and Goldhaber¹¹ ($10^{-6.28}$) and by Ingle *et al.*¹² ($10^{-6.30}$). The value quoted by Fairhall and Erickson¹ for the solubility product of aragonite is correspondingly higher than the original value.

For Plymouth Sound samples at 25 °C, P_{CO_2} values seven to thirteen times those observed at present would be required for the mixed layer to approach the saturation level for calcite, depending on the solubility estimates used (Table 1).

These values are in excess of the most pessimistic predictions made so far for the accumulation of fossil CO₂ in the atmosphere^{3,4}. In some instances the calculated P_{CO_2} values approach the threshold limit value of toxicity (5×10^{-3} atm). They certainly identify as unrealistic those calculations^{3,6} indicating that calcite saturation may be approached at 25 °C following only a 50% increase in the current P_{CO_2} level. The P_{CO_2} values for aragonite saturation at 25 °C are between five and eight times the present value. These facts support my original conclusion² that, at 25 °C, "no account need be taken of the calcium carbonate equilibria since the mixed layer is super-saturated throughout and is likely to remain so". The editorial remarks appended to my original paper² which suggested that "serious environmental effects are not likely in the foreseeable future", do not, however, reflect my own conclusions.

Preliminary experimental¹ (Table 1) and theoretical⁷ results suggest that temperate waters (10 °C) may approach

Table 1 Conditions necessary to achieve saturation with respect to calcite and aragonite for samples taken from Plymouth Sound using different estimates of the mineral solubilities (salinity range 34.69‰ to 34.95‰)

Sample no.	ΣCO_2 (meq l ⁻¹)	CA (meq l ⁻¹)	Mineral	Carbonate solubility ref.	$-\Delta[\text{CO}_3^{2-}]^*$ (mmol l ⁻¹ × 10)	pH ₅ [†]	(P_{CO_2}) _s [‡] (atm × 10 ³)
1‡	2.112	2.410	Calcite	2,14	2.72	7.12	4.41
				7,12	2.61	7.24	3.32
				10,13	2.52	7.33	2.68
			Aragonite	14	2.47	7.37	2.43
				7,12	2.43	7.40	2.26
				10,13	2.17	7.56	1.53
2‡	1.995	2.259	Calcite	2,14	2.38	7.15	3.88
				7,12	2.27	7.27	2.90
				10,13	2.18	7.36	2.33
			Aragonite	14	2.13	7.40	2.12
				7,12	2.09	7.43	1.97
				10,13	1.83	7.59	1.33
3§	2.112	2.282	Calcite	7,12	1.36	7.53	1.46
				10,13	1.19	7.67	1.04
				7,12	1.18	7.68	1.01
			Aragonite	10,13	0.84	7.87	0.63
				7,12	0.95	7.71	0.88
				10,13	0.61	7.90	0.55
4§	1.993	2.140	Calcite	7,12	1.13	7.56	1.28
				10,13	0.96	7.70	0.90
				7,12	0.95	7.71	0.88
			Aragonite	10,13	0.61	7.90	0.55
				7,12	0.95	7.71	0.88
				10,13	0.61	7.90	0.55

*Number of moles of carbonate per litre that must be removed to achieve saturation.

†Subscript S applies to seawater at saturation with respect to the mineral shown if $[\text{Ca}^{2+}] = 10 \text{ mmol l}^{-1}$.

‡Sample adjusted to 25 °C.

§Sample analysed at ambient temperature of 10 °C.

DR WHITFIELD REPLIES — Fairhall and Erickson¹ have objected to several facets of a recent paper² in which I presented the chemical consequences of certain models^{3–6} which have been used to pre-

saturation with respect to aragonite when P_{CO_2} attains approximately three times its present value. The biological implications of such a change are unknown. The attainment of the saturation level may in itself be less significant than the accompanying fall in the pH of the mixed layer.

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Atmospheric halocarbons and stratospheric ozone

LOVELOCK¹ reports atmospheric analysis data for the fluorochlorocarbons (FCCs) CCl_2F_2 and CCl_3F in addition to the chlorocarbons (CCs) CCl_4 , $CHCl_3$, CH_3Cl , CH_2Cl_2 , and CCl_2CCl_2 . The synthetic FCCs are presently of concern as possible catalysts for the destruction of stratospheric ozone. Although Lovelock states that "Molina and Rowland² rightly warn of a potential hazard to stratospheric ozone should the emission of the FCCs continue to grow unchecked", the levels of the CCs reported led him to conclude that "unless there is some special additional effect from the release of chlorine at higher altitudes, there are no grounds for singling out the FCCs as more hazardous than the other halocarbons which penetrate the tropopause".

I do not disagree with that as a statement of the present state of affairs, but I suggest that the conclusion, as stated, is likely to cause confusion as it does not distinguish between the present condition and the probable future values of the FCC/CC ratio, the issue of central concern. The following points seem to have been made by Lovelock or might be reasonably concluded from information presented by him: (1) Present halocarbon levels are at the edge of significant participation in stratospheric ozone destruction cycles. (2) CCl_4 and other CCs are present in far larger amounts than can be explained by reasonable estimates of synthetic sources; the present concentrations of CCs then represent essentially natural levels and are therefore not likely to change significantly in time.

(3) The FCC analysis data suggest, however, that most of the FCC production to date is still present in the troposphere and lower stratosphere. So the sinks for the naturally-occurring CCs do not seem to be significant for the FCCs. For example, use of Lovelock's mole fraction of 1.0×10^{-10} for CCl_2F_2 and an atmospheric scale height of 7.3 km lead to a value of 1.3×10^9 kg for the amount of this compound in a shell 10 km thick around the Earth. This estimate is based on a low value for the tropospheric volume, neglects stratospheric content, and uses an analysis significantly lower than reported elsewhere³. Use of a recent production rate of 0.5×10^9 kg yr⁻¹ and exponential growth with a 3.5-yr doubling period observed back to 1960 (ref. 4) gives an estimate of 2.5×10^9 kg for industrial production to date. Thus a large fraction of the FCCs ever produced can still be accounted for in the atmosphere. The only significant loss process would then seem to be diffusion past the tropopause and subsequent photolysis in the middle stratosphere.

I conclude that the problem is not the consideration that the FCCs might have some special stratospheric reactivity in contrast to the effects of the CCs, as suggested by Lovelock, but rather that the FCC concentrations are likely to continue to grow to critical levels. This has been illustrated by the model calculations of Cicerone, *et al.*⁴. Thus the FCC/CC ratio in the troposphere is most likely to increase steadily with time to a value significantly greater than unity with resulting future consequences for the ozone levels.

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PROFESSOR LOVELOCK REPLIES—If the models of stratospheric ozone destruction by odd chlorine are assumed, then I agree with Chesick that the uninterrupted exponential growth of FCC emissions must eventually establish an atmospheric concentration hazardous on a global scale. In this limited context the only point at issue is when this concentration will be reached. The atmospheric significance of a compound depends on the rate at which it releases chlorine in the stratosphere and this is a function of residence time as well as of abundance. The FCCs have residence times of more than 50 yr whereas the CCs other than CCl_4 have residence times of less than one year. A large fraction of the FCCs entering the stratosphere are returned to the troposphere unchanged, but most of the CCs entering the stratosphere are destroyed there.

I suggested that there might be a large natural chlorocarbon cycle¹. Evidence gathered during the past six months considerably strengthens this conclusion. R. A. Rasmussen (personal communication) has found substantial concentrations of methyl chloride in rural air on the West Coast of the USA. I have confirmed this finding for air coming from the Atlantic with concentrations of methyl chloride now exceeding 10^{-9} by volume. As our investigations proceed, the sum total of atmospheric chlorine carriers discovered grows much more rapidly than does the release of FCCs. The measured ratio of CC to FCC chlorine is now approaching four.

The models of ozone destruction by odd chlorine are plausible and do give cause for concern but in the debate it tends to be forgotten that the models are entirely unconfirmed by direct observations in the stratosphere.

When all these factors are taken into account it does seem that there is time to make those stratospheric measurements from which a realistic upper limit to the concentration of anthropogenic chlorine compounds can be decided. Tropospheric measurements are also needed to complete our understanding of the natural chlorine cycle.

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¹ Lovelock, J. E., *Nature*, **252**, 292-294 (1974).

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Alteration of chicken melanocytes by DNA

LANZA has reported that injection of Harco chicken DNA into 5-d-old White Plymouth Rock embryos permits production of melanin beyond day 14 of incubation, in contrast to uninjected embryos¹. He cites Hamilton's work, in which melanocytes were observed in embryos of all fowl examined, including breeds with white plumage; the melanocytes of white breeds were initially capable of melanin synthesis but degenerated

before hatching². Thus, Lanza states that DNA treatment extends the life span of such melanocytes. Because of related studies in my laboratory, I would like to offer two possible interpretations of Lanza's observations.

We have reported that injection of chicken DNA into White Leghorn eggs induces two types of effects on the embryos, depending on incubation conditions: a lethal effect which may be mediated by a genetic-like mechanism and a survival-enhancing effect involving a non-genetic mechanism³. Both effects are inducible by isologous DNA. Highly polymerised DNA can also enhance mammalian cell viability *in vitro* under conditions excluding a genetic-like mechanism⁴. Such a phenomenon might account for the extended survival of melanocytes in Lanza's system. While we did not observe induction of pigment in more than 200 hatched chickens whose eggs had been injected with non-degraded DNA (either isologous or Rhode Island Red), our experimental procedures differed extensively from Lanza's.

Second, several groups, including ours, have reported induction of melanin in mammalian cells by exogenous DNA⁵⁻⁷. Detailed analysis of our data indicated that our effect was the result of alteration of a regulatory gene instead of a structural gene^{8,9}. This would seem to be the case for Lanza's system if it involved genetic transformation for pigmentation (instead of survival). As noted above, his target cells presumably contained genetic information for melanin production before DNA treatment.

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DR LANZA REPLIES—The points made by Dr Glick are well taken. A survival-enhancing effect similar to that cited³, could indeed have extended the life span of the melanocytes reported.

I would like to point out that all five of the embryos which had patches of pigmented feathers were examined between days 14 and 16 of incubation. Although one of the newborn chicks developed some pigmented feathers, no pigment was observed in the juvenile down of any chickens hatched in earlier experiments. This suggests that all the melanocytes were not altered permanently, and those that were, were too few in most cases to synthesise enough melanin to be detected in the juvenile down of a newborn chicken.

Glick's experimental procedures differ extensively from the experiment reported. Alterations in the embryonic stage would have gone undetected because he observed only hatched chickens. It should be emphasised that the pigmentary genes in the White Leghorns referred

to in Glick's response are considered dominant, whereas those in White Plymouth Rocks are considered recessive¹⁰. I am led to conclude that any genetic transformation would be non-pigmentary and would directly involve the genes responsible for the degeneration of the melanocytes.

Although Glick's analysis of induced melanin synthesis in mammalian cells indicated that the effect was due to a regulatory gene instead of a structural gene⁸, he should not preclude the possibility of a structural gene defect in White Plymouth Rock melanocytes. The lack of pigmentation in most mammalian cells results from a gene-enzyme defect, in which tyrosinase fails to catalyse the reaction in which tyrosine is hydroxylated to dihydroxyphenylalanine. This is not the case, however, with White Plymouth Rocks as Glick stated, the melanocytes in that system were initially capable of melanin synthesis. Therefore genetic transformations involving pigmentation in this breed, would not necessarily be the result of a regulatory gene.

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Schwarzschild orbital topography and high Doppler blueshifts

CHITRE, NARLIKAR AND KAPOOR¹ discuss the high Doppler blueshift of forward light emission from material particles in circular orbit in a Schwarzschild field at and near $r = 3GM/c^2$, which they claim is the radius of the unstable circular orbit (meaning, apparently, "the marginally stable circular orbit").

This supposed effect rests on a basic confusion concerning the orbital topography and energetics in the Schwarzschild field. Outside the event horizon, at $r = 2GM/c^2$, there are three important circular orbits²: the marginally stable circular orbit at $r_{ms} = 6GM/c^2$, inside which there can be no stable circular orbits (Chitre *et al.*¹ mistakenly put this at $r = 3GM/c^2$); the marginally bound circular orbit at $r_{mb} = 4GM/c^2$, inside which there can be no bound circular orbits; and the photon orbit at $r_{ph} = 3GM/c^2$, which has an infinite energy

per unit mass and requires a tangential velocity $v_{tang} = c$. Chitre *et al.*¹ seem to have confused the marginally stable circular orbit with the photon orbit. So it is not surprising that they obtain an infinite blueshift there, since any particle in such an orbit must have $v_{tang} = c$. But, of course, massive particles are barred from such orbits; and those in circular orbits of radii approaching $r = 3GM/c^2$ are not only in an unstable configuration but also highly unbound ($E \gg 1mc^2$). In any realistic physical situation they will all move along outward spiral orbits.

Near $r_{ms} = 6GM/c^2$ particles will make many revolutions, but there will be no net Doppler blueshift, since for these $v_{tang} \approx c/2$. As particles reach $r = r_{ms}$ through, for example, viscous drift, they plunge towards the black hole along spiral orbits. For a test particle moving along such a spiral geodesic, conserving total energy ($E_{ms} = 0.943 mc^2$) and angular momentum ($L_{ms} = 3.46GMm/c$), where m is the mass of the test particle, 4.85 revolutions will be executed between $r = r_{ms}$ and $r = 2GM/c^2$ (W. R. S., unpublished); and the first four revolutions will be between $r = r_{ms}$ and $r = 5GM/c^2$. If dissipative and radiation processes are important, the spiral orbits will have even fewer revolutions. So there seems to be little reason to expect that there could be a thin disk of massive particles executing even approximately circular orbits in the "high blueshift region" near $r = 3GM/c^2$.

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DRS CHITRE, NARLIKAR AND KAPOOR REPLY—We meant what we said in our paper. The orbit in question is close to $r = 3GM/c^2$ as stated, and is not near to $r = 6GM/c^2$ as Stoeger would have us suppose. We are aware that these orbits are unstable and it is not intended that the particle should circulate in such orbits for ever. The circular orbit is meant to be an approximation to the geodesic trajectory of the infalling particle with high energy and small impact parameter, as discussed by Misner *et al.*¹. Those authors have discussed gravitational synchrotron radiation from particles transiting close to $r = 3GM/c^2$. These and related references have already been cited in our paper so no further clarification is necessary.

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¹ Misner, C. W., *et al.*, *Phys. Rev. Lett.*, **28**, 998 (1972).